

Assessment and management of seafood safety and quality

Current practices and emerging issues



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Inset bottom: A variety of fish. ©FAO/FIPM

Assessment and management of seafood safety and quality

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Current practices and emerging issues

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Preparation of this document

In 1994, FAO published the document *Assurance of Seafood Quality* by H.H. Huss (FAO Fisheries Technical Paper No. 334). It was based on a series of lecture notes used at workshops and training activities organized by the FAO/Danish International Development Agency (DANIDA) Training Project on Fish Technology and Quality Control (GCP/INT/391/Den).

By the end of 2000, it had become clear that this document required updating. New ideas and developments, particularly in the presentation of the Hazard Analysis and Critical Control Points (HACCP) concept, needed to be included. In early 2002, FAO asked the same author to prepare an updated and expanded version of the 1993 document. As extensive and significant changes had been made compared with the first document, a new title was chosen: *Assessment and Management of Seafood Safety and Quality*. This updated FAO publication (FAO Fisheries Technical Paper No. 444) appeared in 2004 and was subsequently translated into several languages.

In response to the increasing importance of seafood trade and to the significant changes in the regulatory environment in the last decade, this new and revised FAO Fisheries and Aquaculture Technical Paper has re-examined the whole area of seafood safety and quality, building upon the base provided by FAO Fisheries Technical Paper No. 444. This technical paper focuses on:

- developments in food safety and quality management systems;
- characterization of the food safety hazards in seafoods and seafood quality;
- implementation of management systems to ensure safe and high-quality seafoods.

The study also analyses:

- the regulatory framework that all food business operators (producers, processors, distributors and retailers) must now operate within – at the international, regional and national levels;
- the probable impact of climate change on food safety, focusing on the most important hazards – microbial pathogens and natural toxins from algal blooms;
- the challenges facing developing countries.

Following the same successful formula as used in the previous version, FAO approached professional colleagues from around the world, all eminent in their fields, to contribute to this new version. The contributors are:

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Abstract

This technical paper compiles the state of knowledge on seafood safety and quality with the aim to provide a succinct yet comprehensive resource book to seafood quality and safety managers, including topics on emerging issues such as new pathogens, the impact of climate change on seafood safety, and the changing regulatory framework.

After introductory chapters about world fish production, trade, consumption and nutrition, and about the developments in safety and quality systems, the technical paper devotes a chapter to a detailed review of the hazards causing public health concerns in fish and fish products, covering biological (pathogenic bacteria, histamine, viruses, parasites and biotoxins), chemical (veterinary drugs, industrial organic contaminants, environmental inorganic contaminants and allergens) and physical hazards. This is followed by a chapter on seafood spoilage and quality issues, while a further chapter covers the likely impact of climate change on seafood safety. The latter chapter focuses on impacts on microbiological safety and on harmful algal blooms.

A further chapter provides a detailed coverage of the implementation and certification of seafood safety systems covering risk mitigation and management tools, with a detailed description of the requirements for the implementation of: good hygiene practices and good manufacturing practices; the Hazard Analysis and Critical Control Points (HACCP) system; and the monitoring programmes to control biotoxins, pathogenic bacteria and viruses and chemical pollutants. It concludes with a section on private labelling and certification schemes.

The subsequent chapter details the international framework, covering the World Trade Organization, the Codex Alimentarius Commission, the FAO Code of Conduct for Responsible Fisheries, and the World Organisation for Animal Health. It then presents the regulatory frameworks governing seafood trade in the European Union (Member Organization), the United States of America, Japan, Australia and New Zealand.

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Abbreviations and acronyms

ACC	Aquaculture Certification Council
ADI	acceptable daily intake
ADP	adenosine diphosphate
AHD	1-aminohydantoin
AIDS	acquired immunodeficiency syndrome
ALOP	appropriate level of protection
AMTZ	3-amino-5-morpholinomethyl-1,3-oxazolidin
AMP	adenosine monophosphate
AMS	Agricultural Marketing Service (United States of America)
AOAC	Association of Official Analytical Chemists
AOZ	3-amino-2-oxazolidinone
APFSWG	Working Group on Animal Production Food Safety
AQIS	Australian Quarantine and Inspection Service
AQS	Alaska Quality Seafood
ASP	amnesic shellfish poisoning
ATP	adenosine triphosphate
a_w	water activity
AZA	azaspiracid
AZP	azaspiracid shellfish poisoning
BAP	Best Aquaculture Practices (of the GAA)
BFR	brominated flame retardant
BMP	best management practice
BRC	British Retail Consortium
BSE	bovine spongiform encephalopathy
CAC	Codex Alimentarius Commission
CBAE	cold-blooded animals and environment
CBP	Customs and Border Protection (United States of America)
CCFFP	Codex Committee on Fish and Fishery Products
CCP	critical control point
CCRVD	Codex Committee on Residues of Veterinary Drugs in Foods
CCvD HACCP	Netherlands National Board of Experts – HACCP
CDC	Centers for Disease Control and Prevention (of the United States of America)
CEN	European Committee for Standardization
CEO	chief executive officer
CFP	ciguatera fish poisoning
cfu	colony forming unit
COOL	Country of Origin Labeling

CO ₂	carbon dioxide
Code	FAO Code of Conduct for Responsible Fisheries
COFI	FAO Committee on Fisheries
CPFFP	Code of Practice for Fish and Fishery Products
DAEC	diffuse-adhering <i>E. coli</i>
DAFF	Department of Agriculture, Fisheries and Forestry (Australia)
DAP	defect action point
DBPCFC	double-blind controlled food challenge
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DHA	docosohexaenoic acid
dl-PCB	dioxin-like PCB
DMA	dimethylamine
DNA	deoxyribonucleic acid
DOI	Department of the Interior (United States of America)
DPD	N,N-diethyl-p-phenylene-diamine
DSP	diarrhoeic shellfish poisoning
DTX	dinophysistoxin
EAEC	enteroaggregative <i>E. coli</i>
EC	European Commission
EDTA ₂ Na	ethylene diamine tetraacetic acid disodium
EFSA	European Food Safety Authority
EHEC	enterohaemorrhagic <i>E. coli</i>
EIEC	enteroinvasive <i>E. coli</i>
ELISA	enzyme-linked immunosorbent assay
ENSO	El Niño Southern Oscillation
EPA	eicosapentaenoic acid
EPA	Environmental Protection Agency (United States of America)
EPEC	enteropathogenic <i>E. coli</i>
ETEC	enterotoxigenic <i>E. coli</i>
FBO	food business operator
FDA	Food and Drug Administration (United States of America)
FMI	Food Marketing Institute
FMIA	Federal Meat Inspection Act (United States of America)
FoS	Friend of the Sea
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety Inspection Service (United States of America)
FSMA	Food Safety Modernization Act (United States of America)
FSMS	food safety management scheme
FSO	food safety objective
FVO	Food and Veterinary Office (European Commission)
GAA	Global Aquaculture Alliance
GAP	good aquaculture practice

GATT	General Agreement on Tariffs and Trade
GFSI	Global Food Safety Initiative
GHP	good hygiene practice
GMP	good manufacturing practice
GS1	Global System one
GTP	guanosine triphosphate
HACCP	Hazard Analysis and Critical Control Point (system)
HAB	harmful algal bloom
HAV	hepatitis A virus
HBCD	hexabromocyclododecane
HDC	histidine decarboxylase
HFP	histamine fish poisoning
HIV	human immunodeficiency virus
HPB	histamine-producing bacteria
HPLC	high-performance liquid chromatography
HUS	haemolytic ureamic syndrome
ICMSF	International Commission on Microbiological Specifications for Foods
ICON	Import Conditions database (Australia)
IMP	inosine monophosphate
IQ	intelligence quotient
IFS	International Food Standard
ISO	International Organization for Standardization
ISSC	Interstate Shellfish Sanitation Conference (United States of America)
IUU	illegal, unreported and unregulated (fishing)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LACF	low-acid canned food
LC-MS/MS	liquid chromatography and tandem mass spectrometer
LCn3PUFA	long-chain n-3 polyunsaturated fatty acid
LMG	leucomalachite green
LWE	live weight equivalent
MAP	modified-atmosphere packaging
MPa	megaPascal
MPI	Ministry for Primary Industries (New Zealand)
MPN	most probable number
MRL	maximum residue level
mRNA	messenger ribonucleic acid
MRPL	minimum required performance limit
MSC	Marine Stewardship Council
NaCl	sodium chloride
NACMCF	National Advisory Committee on Microbiological Criteria for Foods

NGO	non-governmental organization
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no observed adverse effect level
NLV	Norwalk-like virus
NRCP	national residue control plan
NSP	neurotoxic shellfish poisoning
NSSP	National Shellfish Sanitation Program (United States of America)
NZFSA	New Zealand Food Safety Authority
OECD	Organisation for Economic Co-operation and Development
OIE	World Organisation for Animal Health
PAS	publically available standard
PBDE	polybrominated diphenyl ether
PBS	phosphate buffered saline solution
PC	performance criterion
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
PCR	polymerase chain reaction
PFAS	perfluorinated alkylated substance
PFCA	perfluorcarboxylic acid
PFGE	pulsefield gel electrophoresis
PFP	puffer fish poisoning
PO	performance objective
POP	persistent organic pollutant
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PRP	Prerequisite programme
PSP	paralytic shellfish poisoning
PTWI	provisional tolerable weekly intake
PTX	pectenotoxin
QAC	quaternary ammonium compound
QC	quality control
QIM	quality index method
QMRA	quantitative microbial risk assessment
RFID	radio frequency identification
RNA	ribonucleic acid
RPL	recommended performance level
RPP	ribosomal protection protein
RTE	ready-to-eat
SE	Staphylococcal enterotoxin

SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	semicarbazide
SIGES	Sistema Integrado de Gestión (Chile)
SIP	Seafood Inspection Program (United States of America)
SOI	Southern Oscillation Index
SOP	standard operating procedure
SPS	Sanitary and Phytosanitary Measures (Agreement)
SQF	Safe Quality Food
SRSV	small round structured virus
SSO	specific spoilage organism
SSOP	sanitation standard operating procedure
SSPO	Scottish Salmon Producers' Organisation
SSSP	Seafood Spoilage and Safety Predictor
STEC	shiga toxigenic <i>E. coli</i>
TBBP-A	tetrabromobisphenol A
TBT	Technical Barriers to Trade (Agreement)
TCBS	thiosulphate-citrate-bile-sucrose (an agar)
TCDD	2,3,7,8-tetrachloro-dibenzo-p-dioxin
TDH	thermostable direct haemolysin
TEF	toxic equivalence factor
TEQ	toxicity equivalent
TMA	trimethylamine
TMAO	trimethylamine oxide
TQM	Total Quality Management
TRH	TDH-related haemolysin
TVB	total volatile bases
TWI	tolerable weekly intake
UNCED	United Nations Conference on Environment and Development
USDA	United States Department of Agriculture
UV	ultraviolet
VCIA	veterinary critically important antimicrobial
VHIA	veterinary highly important antimicrobial
VIA	veterinary important antimicrobial
WHO	World Health Organization
WPS	water phase salt
WTO	World Trade Organization
w.w.	wet weight
WWF	World Wide Fund for Nature
XML	extensible mark-up language
YTX	yessotoxin

1. Introduction

1.1 IMPORTANCE OF SEAFOOD SAFETY AND QUALITY (LAHSEN ABABOUCHE)

Today, food safety remains a major concern facing the seafood industry, and it is a critical component in ensuring food and nutrition security worldwide. The production and consumption of safe food are central to any society, and they have a wide range of economic, social and, in many cases, environmental consequences.

The issue of seafood safety is even more important in view of the growth in international fish trade, which has undergone tremendous expansion in the last three decades, increasing from US\$8 billion in 1976 to a record export value of US\$102.5 billion in 2010. Developing countries play a major role in international fish trade. In 2010, their exports represented 49 percent (US\$42.5 billion) of world fish exports in value and 59 percent (31.6 million tonnes live weight equivalent) in volume.

The well-known food scares of “mad cow disease” and the “dioxin crisis”, and other food safety problems, have forced control agencies to rethink food safety strategies in recent decades, taking a value chain approach and introducing traceability requirements.

In the new millennium, food production and distribution are globalized and even more complex. The advent of emerging pathogens and the impacts of climate change on food safety are adding to this complexity. The media and consumers have developed a much greater interest in food safety issues owing to the continuing incidence of food scares – recent major examples being:

- In Germany, a new strain of *E. coli* linked to bean sprouts infected more than 3 500 people and killed 53.
- In the United States of America, a *Listeria* outbreak resulted in 100 cases and 18 deaths, leading to recalls of about 5 000 freshly cut cantaloupes, while a *Salmonella* outbreak linked to peanut butter resulted in more than 500 cases in 43 states and led to recalls worth US\$1 billion.
- In China, official figures indicate that 6 babies died and 294 000 were made sick from the intentional addition of melamine to various foodstuffs, mainly milk and infant formulas.

The advent of the Hazard Analysis and Critical Control Points (HACCP) system in recent decades has provided a single system that has now been adopted by international bodies and trading countries and regions to control food safety. However, there are important foundations to be put in place before implementing the HACCP system. International organizations have defined the importance of so-called prerequisite programmes, and this clearly differentiates the prerequisite programmes from the HACCP system – something that is always not fully appreciated by processors in many countries. Moreover, various bodies have defined what is required in these “pre-HACCP” operations and, while there is overlap, they do differ. This lack of a universally agreed set of operations prior to implementing HACCP has possibly given rise to the lack of consistency in documentation and implementation of these procedures when compared with the very structured approach offered by the 12 steps of the HACCP system.

More recently, the International Organization for Standardization (ISO) has developed the ISO 22000 family of standards on food safety management systems. It takes the approach of ISO 9001 as a management system, and incorporates the hygiene

measures of prerequisite programmes and the HACCP principles and criteria. In 2008, PAS 220:2008 was developed to cover what were seen to be shortcomings in the prerequisite element of ISO 22000 at the time.

The frameworks for ensuring food safety in the international context are provided by: (i) the World Trade Organization (WTO) under two binding agreements (the Agreement on the Application of Sanitary and Phytosanitary Measures [SPS Agreement] and the Agreement on Technical Barriers to Trade [TBT Agreement]); (ii) the Codex Alimentarius Commission (CAC) through various instruments, for example, the Code of Practice for Fish and Fishery Products and the basic texts on Food Hygiene; and (iii) the FAO Code of Conduct for Responsible Fisheries (the Code), especially under Article 6 (General principles, provisions 6.7 and 6.14) and Article 11 (Post-harvest practices and trade), both of which are of particular relevance to fish trade, safety and quality.

The public health significance of seafood-borne illnesses depends on the likelihood and the severity of the illness. The concept of “risk analysis” has become the method for establishing tolerable levels of hazards in foods in international trade and, equally, within national jurisdictions. In the current international food safety management environment, the risk is expressed as “food safety objectives” in order to achieve what is called an “appropriate level of protection” for populations.

For international fish trade, countries and regions have developed national and regional regulations to control seafood entering or exiting their territories. As more than 70 percent of seafood trade is destined to three main markets (the European Union [Member Organization], the United States of America, and Japan), these markets are important regulatory reference points.

The United States of America has a decentralized system for food safety and quality regulation. There are no fewer than 17 federal government agencies involved in food regulation. The two most important agencies are the Food and Drug Administration of the Department of Health and Human Services, which regulates all food except meat and poultry, and the Food Safety Inspection Service of the Department of Agriculture, which is primarily responsible for meat and poultry. The recent Food Safety Modernization Act of 2011 is now the guiding legislation for improved food safety in the United States of America.

In the European Union (Member Organization), and as the result of a white paper on food safety in 2000, the approach taken in the legislation is to separate aspects of food hygiene from animal health and to harmonize food control across the member countries of the European Union (Member Organization). A key aspect of the legislation is that all food and feed business operators, from farmers and processors to retailers and caterers, have principal responsibility for ensuring that food placed on the market in the European Union (Member Organization) meets the required food safety standards.

Japan has enacted the Food Safety Basic Law, a comprehensive law to ensure food safety to protect the health of the public. In the wake of the development of the basic law and other related laws, Japan has introduced a risk analysis approach to the national food safety control programme work. The Food Safety Basic Law assigns responsibility for risk assessment, and the Food Sanitation Law and other related laws identify those responsible for risk management. The risk assessment is, in practice, conducted by the Food Safety Commission established under the Food Safety Basic Law.

While efforts in the major markets are focusing on a regulatory framework to ensure the safety of consumers, there are implications for the major exporting markets in the developing world. Developing countries have pointed to the challenge presented by these national and regional safety and quality control regimes that vary from one jurisdiction to the next. This multitude of approaches imposes significant costs on

exporters in countries where there is limited capacity to develop comprehensive safety and quality management systems and infrastructures, let alone several different systems to meet diverse import market requirements. Although progress has been made in terms of harmonization, in particular via the WTO and the CAC, it has been slow and more work is required. The concerns expressed by developing countries in relation to public regulation in importing countries are mirrored in their concerns related to private standards for food safety.

Hence, there is a need for continued technical assistance and dissemination of relevant information to developing nations to help them meet the ever-increasing and more complex challenges posed by international markets. It is hoped that this publication will assist governments and industry in developing countries to meet these challenges.

1.2 WORLD SEAFOOD PRODUCTION, UTILIZATION, CONSUMPTION AND TRADE (LAHSEN ABABOUC AND JOHN RYDER)

1.2.1 Fisheries and aquaculture production

World fish production from capture fisheries and aquaculture is very significant for global food security and food trade, providing an apparent per capita food fish supply of 18.8 kg (live weight equivalent [LWE]) in 2011, which is the highest on record. Total production consistently increased from 128 million tonnes in 2002 to 154 million tonnes in 2011 (Table 1).

TABLE 1
World fisheries and aquaculture production and utilization, 2002–2011

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Million tonnes										
PRODUCTION										
Inland										
Capture	8.4	8.6	8.7	9.4	9.8	10.1	10.2	10.4	11.2	11.1
Aquaculture	23.3	24.9	27.2	29.1	31.3	33.4	36.0	38.1	40.9	43.9
<i>Total inland</i>	31.7	33.5	35.9	38.5	41.1	43.4	46.2	48.5	52.1	55.0
Marine										
Capture	82.6	79.7	84.1	83.1	80.4	80.7	79.9	79.6	77.7	82.4
Aquaculture	13.5	14.0	14.7	15.2	16.0	16.6	16.9	17.6	18.1	18.8
<i>Total marine</i>	96.2	93.7	98.8	98.2	96.4	97.3	96.8	97.2	95.9	101.2
<i>TOTAL CAPTURE</i>	91.0	88.3	92.7	92.5	90.2	90.7	90.1	90.0	89.0	93.5
<i>TOTAL AQUACULTURE</i>	36.8	38.9	41.9	44.3	47.3	49.9	52.9	55.7	59.0	62.7
<i>TOTAL WORLD FISHERIES</i>	127.8	127.2	134.6	136.8	137.5	140.7	143.0	145.7	148.0	156.2
UTILIZATION										
Human consumption	100.5	103.6	106.7	109.8	114.5	117.7	120.1	124.0	127.8	131.8
Non-food uses	27.3	23.6	27.9	27.0	23.0	23.0	22.9	21.8	20.2	24.3
Population (billions)	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.8	6.9	7.0
Per capita food fish supply (kg)	16.0	16.3	16.6	16.9	17.4	17.7	17.8	18.2	18.5	18.9

Note: Fishery production data presented in the above table exclude the production for marine mammals, crocodiles, corals, sponges, shells and aquatic plants.

Source: FAO Fisheries and Aquaculture Statistics and Information Branch (2013).

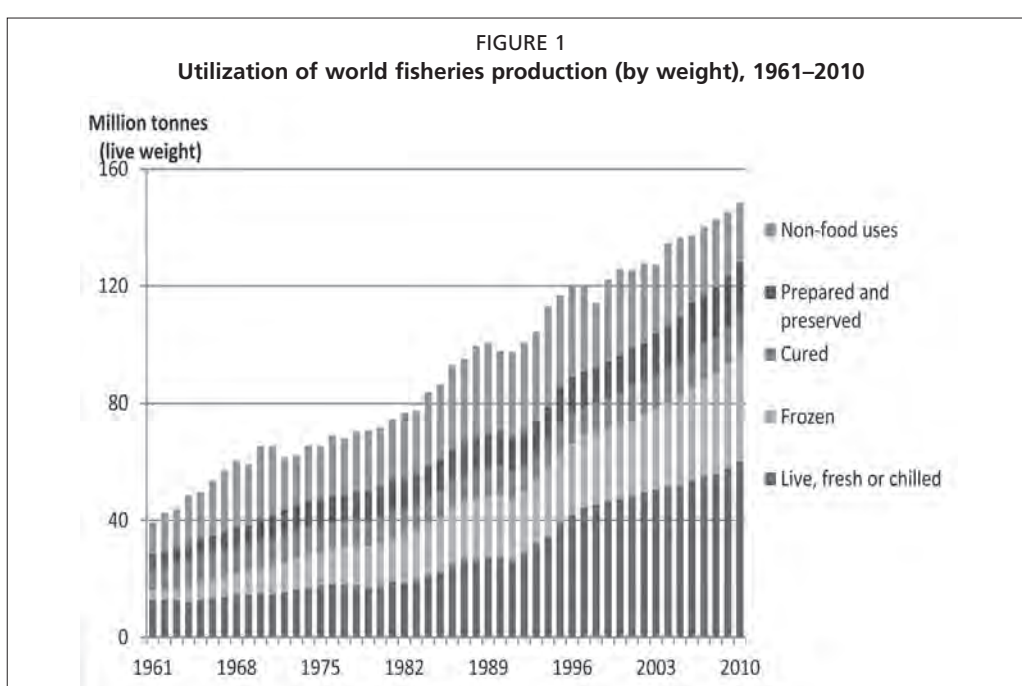
While fish production from wild capture fisheries has fluctuated over the years from 88 million to 93 million tonnes, the demand for fish and fishery products has continued to rise. Consumption has more than doubled since 1973. The increasing demand has been steadily met by a robust growth in aquaculture production, estimated at an average annual growth rate of 8.5 percent in terms of volume in the period 1990–2005. Consequently, global aquaculture production reached 64 million tonnes in 2011.

1.2.2 Fish utilization

Because fish and seafood are perishable, they are often processed to conserve their nutritional properties and prolong their shelf-life. It is estimated that more than 1 200 fish and seafood species are exploited commercially worldwide, with a wide variation in appearance, taste and price, although their nutritional attributes are broadly similar, particularly with reference to their protein content (OECD, 1995).

In the period 2002–2011, 100–131 million tonnes, representing on average more than 80 percent of yearly world fish production, were used for direct human consumption (Table 1). The remaining 20 percent were destined for non-food products, in particular for the manufacture of fishmeal and fish oil.

Figure 1 shows the evolution of the utilization of world fisheries and aquaculture production between 1961 and 2010.



Note: Fishery production data presented in the above figure exclude marine mammals, crocodiles, corals, sponges, shells and aquatic plants.

Source: FAO Fisheries and Aquaculture Statistics and Information Service (2012).

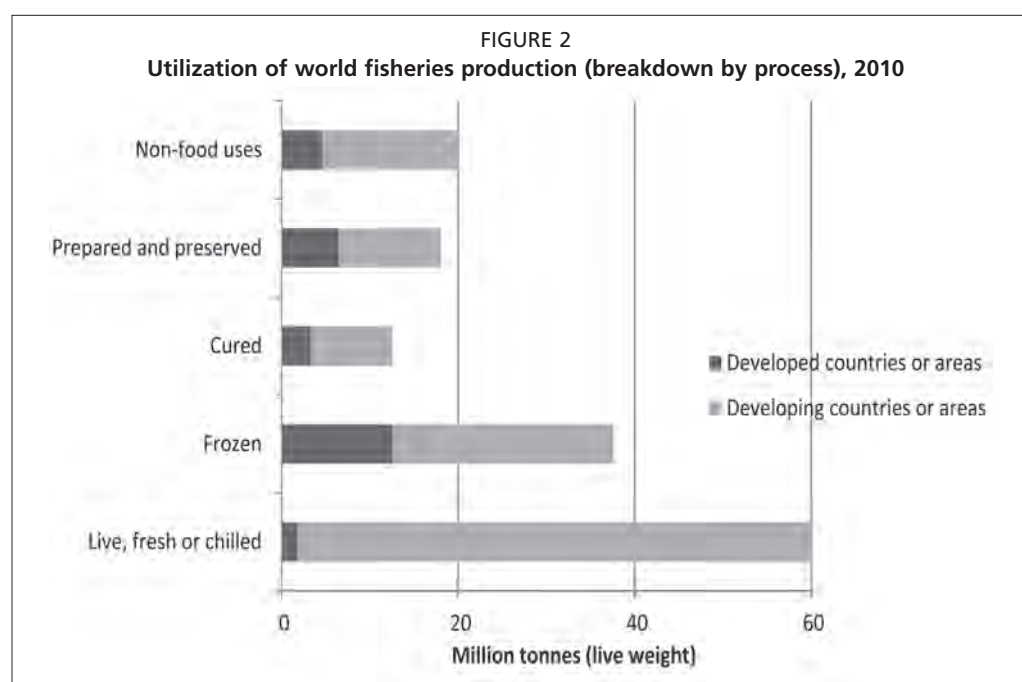
In 2010, 40 percent of the fish destined for human consumption was in live and fresh form, which can be the most preferred and highly priced product form (except for high-value smoked fish). Sixty percent (88 million tonnes) of the world's fish production underwent some form of processing by freezing, curing, canning or extraction of fishmeal and/or fish oil. Seventy-seven percent (68 million tonnes) of this processed fish was used for direct human consumption in frozen, cured and prepared or preserved form, and the rest for non-food uses.

Figure 1 shows that the proportion of fish marketed in live/fresh form worldwide increased more significantly over the years compared with other products. Live/fresh fish quantities increased from an estimated 18 million tonnes in 1980 to 28 million tonnes in 1990, 47 million tonnes in 2000 and 60 million tonnes in 2010, representing an increase in its share of total production from 25 percent in 1980 to 40 percent in 2010. For longer shelf-life, freezing represents the main method of processing fish for food use, accounting for 55 percent of total fish processed for human consumption in 2010, followed by canning (26 percent) and curing (18 percent). In fact, the volume of fish destined for curing has changed only marginally in the last 25 years. A similar trend

is seen for fish destined for canning, which stagnated at about 11–12 million tonnes for many years, albeit showing a greater increase in the period 2000–2010, going from 12 million to 18 million tonnes per annum.

Across the world, developing countries prepare and/or process a large volume, estimated at 120 million tonnes, or about 80 percent of the global fish production in 2010, of which 49 percent, representing 56 percent of their fish food utilization, was utilized as fresh/live, whereas developed countries used frozen fish most, 43 percent of their total fish utilization and 56 percent of their fish food. By comparison, the share of frozen products was 20 percent of their total fish utilization (24 percent of fish food) in developing countries, although in absolute terms it was almost double that in developed countries by volume. Fish curing and the production of fishmeal and fish oil is mostly done in developing countries, whereas canning is significant in both developed and developing countries, although greater volumes are canned in developing countries (Figure 2).

However, in many developing countries with hot climates, quality deterioration and significant post-harvest losses occur because of inadequate use of ice, poor access to roads and electricity, and inadequate infrastructure and services in physical markets. Market infrastructure and facilities are often limited and congested, increasing the difficulty of marketing perishable seafood. This, together with well-established consumer habits, explains why fish production is utilized in such countries mainly in live/fresh form or processed by smoking, drying or fermentation. Given the limited cold chain in many developing countries and the large volumes distributed as fresh fish, it is likely that their quality and nutritional benefits deteriorate before consumption. Likewise, fish destined for curing are, in several developing countries, often made of unsold or substandard-quality fresh fish, with the same negative consequences on quality and nutritional benefits. This highlights the increasing need for improved appropriate and cost-effective technologies to preserve fish quality and nutritional benefits in developing countries.



Note: Fishery production data presented in the above figure exclude marine mammals, crocodiles, corals, sponges, shells and aquatic plants.

Source: FAO Fisheries and Aquaculture Statistics and Information Service (2012).

In terms of products, the utilization and processing of fish production have diversified significantly in the last two decades, fuelled by changing consumer tastes and advances in technology, packaging, logistics and transport. These changes have included improvements in storage and processing capacity, together with major innovations in refrigeration, ice-making, food-packaging and fish-processing equipment. Modern vessels now incorporate improved equipment and are able to stay at sea for extended periods. This has permitted the distribution of more fish in live or fresh form. Moreover, improved processing technology enables higher yields and results in more fish food from the available raw material.

The practice of outsourcing processing is increasing significantly, its extent depending on the species, product form, and cost of labour and transportation. For example, whole fish from European and North American markets are sent to Asia (China in particular, but also India and Viet Nam) for filleting and packaging, and then re-imported, although these trends are slowing or even reversing in some cases. In Europe, smoked and marinated products are being processed in Central and Eastern Europe, in particular in Poland and in the Baltic countries. European shrimp is peeled in North Africa, and European or American tuna is canned in many African and Latin American countries. The further outsourcing of production to developing countries is restricted specifically by certification requirements, especially sanitary requirements, which can be difficult to meet.

Finally, about 13 percent of world fish production was used for non-food products in 2010, with the bulk (about 70 percent) being converted into fishmeal and fish oil. The remainder, mainly consisting of low-value fish, is largely utilized as direct feed in aquaculture and livestock. In 2009, the quantity of fish used as raw material for fishmeal was about 17.9 million tonnes, down 20 percent from 2005 and well below the peak levels of more than 30 million tonnes recorded in 1994. The bulk of the fish products used for non-food purposes came from natural stocks of small pelagics. The decrease in fishmeal production in the past decade has been irregular, its considerable fluctuations mainly reflecting annual variations in catches of small pelagics, especially anchoveta.

1.2.3 Fish consumption

Fish is highly nutritious, rich in micronutrients, minerals, polyunsaturated fatty acids and proteins, and represents a valuable supplement in diets lacking these nutrients, essential vitamins and minerals. In many countries, especially developing countries, the average per capita fish consumption may be low, but, even in small quantities, fish can significantly improve the quality of dietary proteins by complementing the essential amino acids that are often present only in low quantities in vegetable-based diets.

In the past four decades, fish consumption has undergone major changes. World apparent per capita fish consumption has increased steadily, from an average of 9.9 kg in the 1960s to 14.4 kg in the 1990s and 18.8 kg in 2011 (Table 1). However, there are large variations across countries and regions of the world, reflecting different eating habits and traditions, availability of fish and other foods, prices, socio-economic levels, and seasons. As a consequence, per capita apparent fish consumption can vary from less than 1 kg in one country to more than 100 kg in another. Differences are also evident within countries, with consumption usually higher in coastal areas.

Of the 124 million tonnes available for human consumption in 2009¹, consumption was lowest in Africa (9.7 million tonnes, with 9.7 kg per capita), while Asia accounted for two-thirds of total consumption, including 43.2 million tonnes consumed outside China (15.5 kg per capita), and 42.8 million tonnes in China alone (32.1 kg per capita).

¹ FAO Food Balance Sheets of fish and fishery products, Statistics and Information Service of the Fisheries and Aquaculture Department, August 2013.

The corresponding figures for Oceania, North America, Europe and Latin America and the Caribbean were 24.5, 22.0, 22.2 and 9.8 kg per capita, respectively.

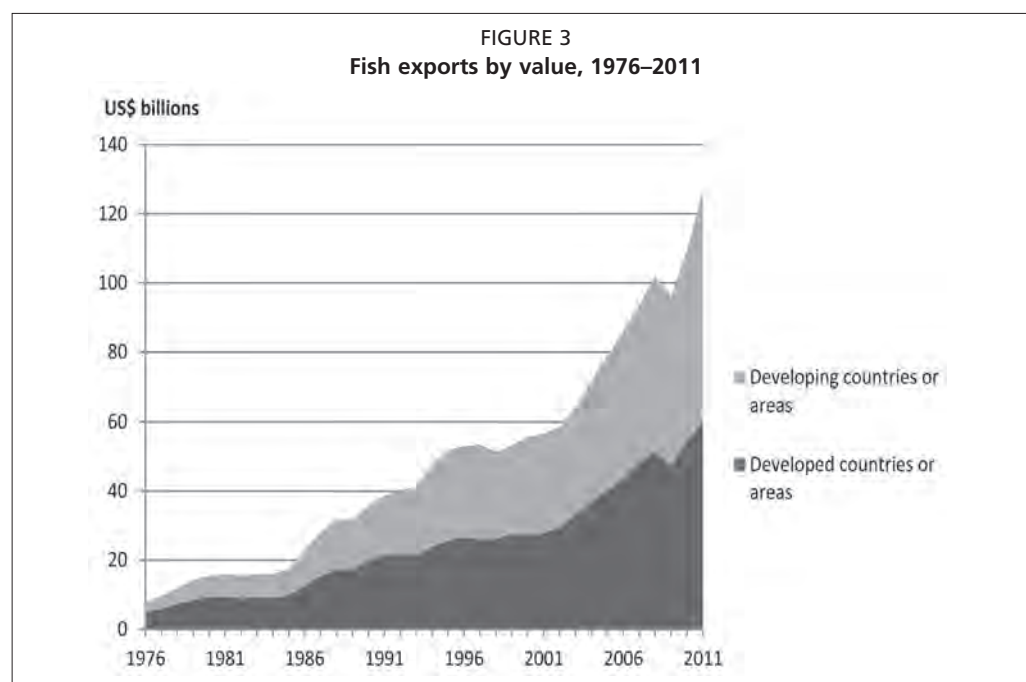
The contribution of aquaculture to fish food supply has increased significantly to reach 48 percent in 2011, up from a mere 6 percent in 1970. This trend is projected to continue, with the contribution of aquaculture to fish food supply estimated to reach 60 percent by 2020, if not before.

Aquaculture production is pushing the demand for and the consumption of several freshwater species, such as tilapia and catfish (including *Pangasius* species) as well as for high-value species, such as shrimps, salmon and bivalves. Since the mid-1980s, these species have shifted from being primarily wild-caught to being primarily farmed, with a decrease in prices and a strong increase in commercialization. Aquaculture has also had a major role in terms of food security in several developing countries, particularly in Asia, with significant production of low-value freshwater species such as carps, mainly for domestic consumption (De Silva, 2008).

1.2.4. Fish trade

Total world trade of fish and fishery products has undergone tremendous development in the last three decades, increasing from a mere US\$8 billion in 1976 to US\$126 billion in 2011 (Figure 3).

A specific feature of the trade in fish is the wide range of product types and participants. In 2006, 194 countries reported exports of fish and fishery products, of which 97 were net exporters. Export value expanded at an average annual rate of 5 percent in the period 1996–2008, although 2009 saw a decline with a rebound in 2010/11.



Note: Fishery production data presented in the above figure exclude marine mammals, crocodiles, corals, sponges, shells and aquatic plants.

Source: FAO Fisheries and Aquaculture Statistics and Information Service (2012).

Developing countries play a major role in international fish trade. As shown in Figure 3, the shares of export value between developed and have remained fairly equal over the years. In 2006, exports from developing countries represented 49 percent (US\$42.6 billion) of world fish exports in value and 59 percent (31.6 million tonnes

LWE) in volume. In 2009, the share of developing countries in total fishery exports was, for the first time, more than 50 percent by value (50.5 percent) and this rose to 53 percent in 2011. An important share of developing country exports consists of fishmeal (typically about 35 percent by quantity, but only 5 percent by value). Similarly, they contributed about 70 percent in volume of world non-food fishery exports and have been significantly increasing their share of fish export volumes destined for human consumption. Developing countries rely on the markets of developed countries, not only as outlets for their exports, but also as suppliers of their imports for local consumption (mainly low-priced, small pelagics as well as high-value fishery species for emerging economies) or for their processing industries. In recent years, in value terms, about 40 percent of fish imports by developing countries have originated from developed countries. In fact, because of outsourcing, several developing countries are importing increasing quantities of raw material for further processing and re-export to developed countries. Likewise, fishery exports of developing countries are gradually evolving towards further value-added products and high-value live fish.

Viet Nam became the fourth major exporter of fish and fishery products in 2008 (after China, Norway and Thailand). In value terms, shrimp continues to be the most important commodity traded, accounting for 15.0 percent of the total value of internationally traded fish products in 2009, followed by salmon and trout with a share of 14.0 percent. A decade ago, the respective shares were 20 percent and 10 percent. Even if the trade statistics collected by countries do not distinguish between the farmed or wild origin of the fishery species, it is evident that aquaculture is having an increasing relevance in traded products.

Net export revenues of fish and fish products (i.e. the value of fish exports minus the value of fish imports) are particularly important for many developing countries, being higher than those of many other agricultural commodities such as rice, meat, sugar, coffee and tobacco (Table 2). The net exports of fish have increased significantly in recent decades, growing from US\$10.2 billion in 1990 to US\$18.3 billion in 2000 and US\$28.2 billion in 2010.

TABLE 2
Net exports of selected agricultural commodities by developing countries

Commodity	Net exports of developing countries in US\$ billions		
	1990	2000	2010
Fish	10.2	18.3	28.2
Coffee	6.4	7.5	14.8
Natural rubber	2.1	1.9	10.2
Cocoa	2.4	1.8	6.8
Sugar	2.6	0.5	4.0
Banana	2.2	2.2	3.7
Tea	1.2	1.1	2.8
Tobacco	0.4	-0.5	1.2
Rice	-0.7	-0.6	0.1
Meat	-0.3	-2.2	-2.1

World imports of fish and fish products reached a new record of US\$108 billion in 2008, up 95 percent since 1998. However, that figure dropped to US\$100 billion in 2009. With stagnant domestic fishery production and growing demand, developed markets rely on imports and/or on aquaculture to cover a growing share of internal consumption. In total, developed countries accounted for 80 percent of imports in terms of value but only 62 percent in terms of quantity, indicating the higher unit value of products imported by developed countries, with Japan, the United States of America and the European Union (Member Organization) being the leading importers.

About 50 percent of the import value of developed countries originates from developing countries. At present, the main obstacles to increased exports from developing countries are stringent and increasing requirements for food safety, animal health, environmental and social standards. This has led to the emerging dominance of large retail and restaurant chains that increasingly impose private standards and labels on suppliers, making it more difficult for small-scale fish producers to enter international markets.

1.3 FISH IN NUTRITION AND HEALTH (DAVID JAMES)

The contribution of fish to overall food security is increasingly being recognized, both as a source of fish as food and as income to support sustainable livelihoods. Fisheries also create jobs as well as contribute to economic growth and development.

Less highlighted is the crucial role that fish and fishery products play in nutrition and as a source of nutrients of fundamental importance not readily found in other foods. Seafood provides high-quality protein, minerals, essential trace elements, fat-soluble vitamins (vitamin D) and essential fatty acids, particularly long-chain n-3 polyunsaturated acids (LCn3PUFAs). Although most of these nutrients can be obtained from other sources, seafood is a palatable and convenient source.

From a protein consumption perspective, fish accounts for 16.6 percent of the global population's intake of animal proteins and 6.4 percent of all proteins consumed. Globally, fish provides about 2.9 billion people with almost 20 percent of their average per capita intake of animal protein, and 4.2 billion people with 15 percent of such proteins.

From a human health perspective, there is convincing evidence – from extensive prospective cohort studies and randomized trials in humans, together with supportive retrospective, ecological, metabolic and experimental animal studies – that seafood consumption reduces the risk of death from coronary heart disease and that consumption by women reduces the risk of suboptimal neurodevelopment in their offspring. These benefits are attributed to two specific LCn3PUFAs: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

However, along with the benefits, there are attendant risks in terms of food-borne disease, infestation with parasites or dangerous levels of toxic substances (e.g. biotoxins, heavy metals or dioxins). It is a fact that life is not risk-free, and the recognition by food safety agencies that this applies also to food supply has introduced a fundamental change in the approach to the safety and quality of the food chain. Indeed, this publication introduces and explains, in depth, a risk-based inspection system for controlling the safety and quality of the seafood supply. The concept of risk analysis can also be extended to a qualitative, or a quantitative, evaluation of the benefits and risks of seafood consumption.

As a result of the increasing debates, well reported in the media, on how much fish should be eaten and by whom, or even if fish should be eaten at all, the CAC requested FAO and the World Health Organization (WHO) to organize an expert consultation on the risks and benefits of fish consumption in an attempt to balance the equation by the application of sound science. The request was specifically for a comparison of the health benefits with the health risks associated with the contaminants methylmercury and dioxins. Seventeen international experts in the fields of nutrition and toxicology, supported by resource persons, met in Rome in January 2010 to discuss the issues and produced a comprehensive report (FAO/WHO, 2011a). The significant conclusions were:

- Among the general adult population, consumption of fish, particularly fatty fish, lowers the risk of mortality from coronary heart disease. There is an absence of probable or convincing evidence of risk of coronary heart disease associated with methylmercury. Potential cancer risks associated with

dioxins are well below established coronary heart disease benefits from fish consumption.

- When comparing the benefits of LCn3PUFAs with the risks of methylmercury among women of childbearing age, fish consumption lowers the risk of suboptimal neurodevelopment in offspring compared with the offspring of women not eating fish in most circumstances evaluated.

The experts went on to develop a methodology for a quantitative risk-benefit comparison that could be extended to cover other situations where sufficient experimental data are available – the expert consultation also called for the creation of international databases on seafood composition. The methodology could also be extended to the presentation of other risk-benefit comparisons in graphic form as an aid to risk-benefit communication.

In the first case, they compared the benefits from LCn3PUFA intake on neurodevelopment in the offspring of mothers consuming fish in terms of intelligence quotient (IQ) points gained, with the risks of loss of IQ points from methylmercury intake. The second comparison was of changes in mortality from consuming fish with different LCn3PUFA and dioxin contents in terms of lives lost through dioxin-induced cancers with lives saved by reduction in coronary heart disease. Both scenarios strongly support the benefits of fish consumption under almost all circumstances, except where the contaminant levels are excessive or the LCn3PUFA content is very low.

Tables 3 and 4 illustrate the approach taken by the expert consultation. The example in Table 3 is based on data from Europe, North America and Japan and shows the species categorized by LCn3PUFA and total mercury content. Cells shaded yellow indicate fish species that might pose a net risk if consumed four times a week, the remaining species pose no risk if consumed four times a week. A similar table could be developed and adapted to other regions as a tool to provide advice to populations on fish consumption.

TABLE 3

Classification of the content of LC-PUFAs (EPA + DHA) by total mercury content in various finfish and shellfish

	EPA + DHA concentration			
	Less than 3 mg/g	Between 3 and 8 mg/g	Between 8 and 15 mg/g	Greater than 15 mg/g
Mercury concentration	Less than 0.1 µg/g Fish: butterflyfish; catfish; Atlantic cod; Pacific cod; Atlantic croaker; haddock; pike; European plaice; pollock; saithe; sole; tilapia Shellfish: clams; cockle; crawfish; cuttlefish; oysters; periwinkle; scallops; scampi; sea urchin; whelk	Fish: flatfish; John Dory; perch, ocean and mullet; sweetfish; wolf fish Shellfish: mussels; squid	Fish: redfish; Atlantic salmon, (wild); Pacific salmon, (wild); smelt Shellfish: crab, spider; swimcrab	Fish: anchovy; herring; mackerel; rainbow trout; Atlantic salmon, (farmed); sardines; sprat Fish liver: Atlantic cod, (liver); saithe (liver) Shellfish: crab (brown meat)
	0.1–0.5 µg/g Fish: anglerfish; catshark; dab; grenadier; grouper; gurnard; hake; ling; lingcod and scorpionfish; Nile perch; pout; skate/ray; snapper, porgy and sheepshead; tuna, yellowfin; tusk; whiting Shellfish: lobster; American lobster	Fish: bass, freshwater; carp; perch, freshwater; scorpion fish; tuna; tuna, albacore Shellfish: crab; lobster, Norway; lobsters, spiny	Fish: bass, saltwater; bluefish; goatfish; Atlantic halibut, (farmed); Greenland halibut; mackerel, horse; Spanish mackerel; seabass; seabream; Atlantic tilefish; tuna, skipjack	Fish: eel; mackerel, Pacific; sablefish
	0.5–1 µg/g Fish: marlin; orange roughy; tuna, bigeye	Fish: mackerel, king; shark	Fish: alfonsino	Fish: Pacific tuna, bluefin
	Greater than 1 µg/g	Fish: swordfish		

Note: Cells shaded grey indicate fish species that might pose a net risk if consumed four times a week, the remaining species pose no risk if consumed four times a week.

As another example, Table 4 shows IQ points lost and gained by a child as a result of fish consumption by the mother during pregnancy, when four servings of 100 g each are consumed per week. The numbers in the upper row in each cell are estimates of IQ points lost from methylmercury exposure, with the lower value of the two numbers based on the central estimate, and the higher value calculated using a more conservative value, the upper-bound estimate. The numbers in the lower row in each cell are estimates of IQ points gained from the mother's consumption of DHA. The maximum positive effect from DHA on IQ was estimated at 5.8 points. Cells shaded yellow represent the estimates where the net effect on child IQ, using the upper-bound estimate for methylmercury, is negative. If the central estimate for methylmercury is used, the net effect on child IQ will be positive for all species consumed even at frequencies of more than seven times a week.

TABLE 4
IQ points lost and gained by a child as a result of fish consumption by the mother during pregnancy, when four servings, of 100 g each, are consumed per week

4 servings per week		EPA + DHA concentration				
		Less than 3 mg/g		Between 3 and 8 mg/g	Between 8 and 15 mg/g	Greater than 15 mg/g
		Median	2	5.5	11.5	20
Methylmercury concentration	Less than 0.1 µg/g	0.05	–0.08, –0.31	–0.08, –0.31	–0.08, –0.31	–0.08, –0.31
			+3.1	+5.8	+5.8	+5.8
	0.1–0.5 µg/g	0.3	–0.48, –1.9	–0.48, –1.9	–0.48, –1.9	–0.48, –1.9
			+3.1	+5.8	+5.8	+5.8
	Between 0.5–1 µg/g	0.75	–1.2, –4.7	–1.2, –4.7	–1.2, –4.7	–1.2, –4.7
			+3.1	+5.8	+5.8	+5.8
	Greater than 1 µg/g	1.5	–2.4, –9.3	–2.4, –9.3	–2.4, –9.3	–2.4, –9.3
			+3.1	+5.8	+5.8	+5.8

Note: Cells shaded grey represent the estimates where the net effect on child IQ, using the upper-bound estimate for methylmercury, is negative.

This expert consultation provided some evidence-based guidance on seafood consumption and provided methodologies that can be adopted in all parts of the world, given an availability of the appropriate data; hence, the call for international databases on seafood composition to be more widely developed.

2. Developments in food safety and quality systems (Lahsen Ababouch and Iddya Karunasagar)

2.1 HISTORICAL BACKGROUND

Evidence from early historical writings dating back to ancient Assyrian, Egyptian, Greek and Roman times indicates that governing authorities were already concerned with food control and consumer protection. For example, the Romans had a well-organized state food control system to protect consumers from frauds and bad produce. Likewise, in Europe during the Middle Ages, individual countries passed laws concerning the quality and safety of various foods. A major change took place in Europe following the industrial revolution in the nineteenth century. The associated demographic changes resulting from urban development created a massive demand for food that could be processed and stored. This was the start of the modern food processing industry. In the early days, there were many examples of food adulteration, leading to demands for a more systematic system of food control.

As a result, in the latter part of the nineteenth and early part of the twentieth century, important developments in food safety and quality were achieved. These were mainly stimulated by the discovery of microbiology and of major developments in food chemistry. Several studies linked specific agents to epidemics of diseases and documented routes by which these agents can be transmitted to humans, including through foods and water (Gorham, 1970). This enabled major advances in public health to significantly reduce the burden of a number of devastating epidemic diseases.

These achievements were consolidated further during the second part of the twentieth century to accompany the rapid developments and progress in many developed countries in food production, preservation and distribution.

While, in the 1950s, many countries were primarily concerned with securing supply to overcome post-war scarcity, the 1960s was a decade of change with the expansion of modern techniques for processing, preservation, packaging, storage and distribution. This introduced new food safety challenges and required improved hygiene and food control.

In the 1970s, farmers relied to a greater extent on pesticides to protect crops, and additives and flavouring agents integrated the food chain, as localized production declined and large-scale food manufacture grew. These chemicals needed to be regulated and proper enforcement of the regulations was required.

In the 1980s, globalization of food trade took off, with more food products crossing national and continental borders. At the same time, several food scares, caused by bacteria (e.g. *Salmonella*) and chemical contamination (e.g. mycotoxins), increased the importance of food safety as an issue of major public concern.

This concern was exacerbated in the 1990s because of “mad cow disease” and the “dioxin crisis”, which forced regulators to revise food safety strategies – integrating the various components of the value chain and introducing traceability requirements.

In the first decade of this millennium, food production and distribution became more globalized and complex, market choices grew even wider, other food scares emerged globally, and the media and consumers developed greater interest in food

safety, ethical practices, and the environmental and social impacts of food production and distribution.

In parallel, further globalization of supply chains, vertical integration through the use of direct contracts between suppliers and retailers and the expansion of supermarkets in food retailing, both nationally and internationally, has led the retail sector to adopt various private standards and certification schemes. This responds to the increasing influence and concerns of civil society related to health, social and environmental issues of fisheries and aquaculture. By so doing, the retail sector hopes to address the legal requirements of companies to demonstrate “due diligence” in the prevention of food safety risks, to attend to the growing need for “corporate social responsibility” and to minimize “reputational risks”.

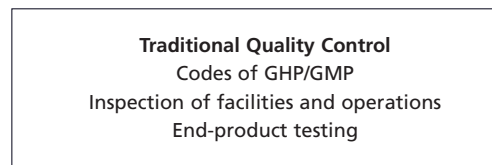
The developments in food production, preservation and distribution that have taken place in the last 60 years have required advances and parallel developments in food engineering, science, technology, safety and quality. Better knowledge of the composition of foods, the functionality of their major and micro constituents and nutrients, their quality, quality changes and associated hazards and the advent of more sensitive and rapid analytical methods have enabled the development of various safety and quality systems and better characterization of foods and their risk categories. Expansion of the food industry and food distribution systems across borders and continents has required the development of quality assurance systems to support business-to-business contractual agreements and verification of conformity of food supplies with the specifications. At the same time, the development of bilateral, regional and multilateral trade agreements has brought about changes in national and supranational food control systems to harmonize requirements and procedures. A major breakthrough came about with the creation of the WTO and the enactment of two international agreements on SPS measures and on TBT. These two agreements established the need to develop SPS measures based on science, in particular assessment of risk, and to promote international harmonization and equivalence of food standards and control systems to facilitate food trade.

Concurrently, government and industry, in collaboration with academia and research institutions, worked on the development of codes of good agriculture, hygienic and manufacturing practices and preventive food safety and quality systems. The food control authorities concentrated their efforts on the inspection of facilities and practices (to ensure adherence to established codes) and on end-product testing to confirm safety of the products and identify the risks. However, industry was ahead of food control agencies in applying more preventive systems. For example, the HACCP system was initiated and adopted by industry in the 1970s, but it was only in the 1990s that most of the food control agencies adopted it into their regulatory framework, and enforcement of regulatory HACCP implementation became effective only in the late 1990s.

Most importantly, the efforts of the industry and food control authorities were not harnessed in a synergistic way until the event of regulatory HACCP food control systems. Much still needs to be done in this respect to promote complementary systems that will enable the control and prevention of food safety hazards at source along the supply chain and decrease the reliance on end-product sampling and testing.

2.2 TRADITIONAL SAFETY AND QUALITY CONTROL

As stated above, food safety and quality control programmes have been based on establishing effective hygiene control and monitoring performance. In the past, confirmation of safety and quality was achieved by end-product testing. Control of hygiene was by inspection of facilities to assess adherence to established and generally accepted codes of good hygiene practice (GHP) and of good manufacturing practice (GMP).



Codes of GHP/GMP and inspection of facilities and operations are still the basis of food hygiene. End-product testing relies on sampling products and subjecting them to testing for safety and quality attributes. The number, size and nature of the samples taken for analysis greatly influence the reliability of the results. In some instances, it is possible for the analytical sample to be truly representative of the “lot” sampled. This applies to liquids such as milk and water that are usually thoroughly mixed. However, in cases of lots or batches of food, this is not the case, and a food lot may easily consist of units with wide differences in (microbiological) quality. Even within the individual unit (i.e. a retail pack), the hazard (i.e. the presence of pathogens) can be very unevenly distributed and the probability of detection may be very low.

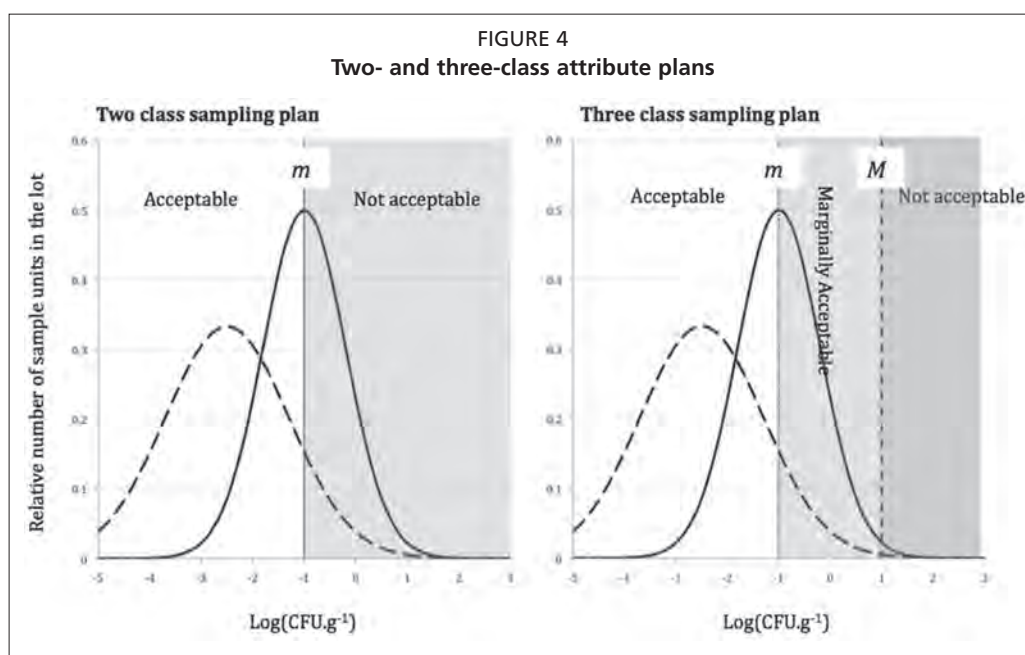
An attributes sampling plan is based on assessment of the number of samples that satisfy some criterion or attribute of the product, e.g. absence of *Salmonella* in 25 g of product, or that the number of *L. monocytogenes* is < 100 colony forming units (cfu)/g, or that histamine levels are ≤ 20 parts per million (ppm). Such a plan is characterized by three elements: n (the number of sample units drawn), c (the maximum allowable number of samples that exceed the criterion) and m the criterion, or attribute, that is being assessed. Thus, in a two-class attributes sampling plan, each sample unit is classified as either “acceptable” or “non-acceptable”. In many cases, the presence of a pathogen (e.g. *Salmonella*) in a specified volume of material sampled from the lot (e.g. 25 g) would be unacceptable. In other cases, m is a number of colony forming units, or other measure of cell density, that differentiates an acceptable from an unacceptable result. The two-class sampling plan will reject a “lot” if more than c out of n samples tested exceed the criterion or attribute.

In a three-class sampling plan, a fourth element is considered, termed M . M is usually a numerical limit that, if exceeded in any sample, causes the entire lot to be rejected. M is always higher than m . In three-class plans, samples with microbial loads in excess of m but less than M are considered to be of “marginal” quality or safety. Figure 4 shows both types of sampling plans.

In addition to diverting important resources, end-product sampling and testing presents many shortfalls, not the least giving a sensation of “being in control” and creating a strong but false sense of security. For example, depending on the sampling plan used for inspecting a lot, the probability of acceptance of the lot will depend on the percentage of defective units in the lot, on the number of samples drawn (n) and the maximum allowable number of defective samples (c). Assuming a lot with 1 percent defective units, a sampling plan with $c = 5$ and $n = 0$, the probability of accepting the lot is $P = C^5_0 (0.99)^5 (0.01)^0 = (0.99)^5 = 0.951$.

Table 5 was constructed using the same method of calculation for different combinations of percentage defective, n and c . It shows that testing of foods offers very little protection even when large numbers of samples are drawn. With 1 percent defective units in the lot, drawing 60 samples, which is usually not feasible on a lot-by-lot basis and not economical for destructive sampling, yields a probability of acceptance equal to 54.7 percent. Assuming 100 lots of 10 000 units each, thus 100 defective units in each lot, even with a sampling plan of $n = 60$ and $c = 0$, more than 54 lots will be accepted because no defective units will be found in their samples of 60 each. To decrease the probability of acceptance, more than 3 000 or 5 000 units would need to be sampled and tested in order to detect a 1 percent defect rate with

95 percent or 99 percent probability (to accept the lot with 5 percent or 1 percent probability).



Notes: The figure on the left represents a two-class sampling scheme in which the attribute, or criterion, (m) is whether the samples exceed 1 cell per 10 g or not. The distributions of log(cell numbers) in two lots is shown, one (solid line) with an average concentration of -1.0 ± 0.8 (SD) log(cfu/g) and one (dotted line) with an average contamination of -2.5 ± 0.8 (SD) log(cfu/g). In the figure, the shaded area shows the proportion of the samples that would be expected to exceed the criterion, and that a greater proportion of the -1.0 ± 0.8 (SD) log(cfu/g) distribution will exceed the criterion. The figure on the right depicts the same distributions with a three-class sampling plan applied in which samples that exceed m are considered marginally acceptable, while samples that exceed M are considered totally unacceptable. Usually, if any sample exceeds M , the lot, as a whole, fails.

Source: Based on ICMSF (2002).

TABLE 5

Effect of lot quality (percentage defective in a lot) on the probability of acceptance for different two-class sampling plans (based on EC, 1998)

Percentage defective samples in lot	Probability of acceptance (%) given sampling plans with a total of n samples when none of the samples is permitted to test "positive" (i.e. when $c = 0$)			
	$n = 1, c = 0$	$n = 5, c = 0$	$n = 10, c = 0$	$n = 60, c = 0$
1	99.0	95.1	90.4	54.7
2	98.0	90.4	81.7	30.0
5	95.0	77.4	59.9	4.6
10	90.0	59.1	34.9	0.18
20	80.0	32.8	10.7	0.00015

Source: Based on EC (1998).

Consequently, even the most elaborate sampling and testing of end product, although unrealistic and uneconomical for routine testing, cannot guarantee safety of the product. There is no way to avoid some degree of risk and error in each acceptance and each rejection of lots unless the entire lot is tested, in which case no edible food will be left for sale.

Furthermore, when the distribution of contaminants in units is heterogeneous, the probability of detection is even lower (Table 6).

TABLE 6

Detection probabilities for end-product testing (presence/absence) of 25 g samples of milk powder contaminated with *Salmonella*

	Contamination rate	Number of random samples	Probability of detection ¹
Homogeneously contaminated	5 cells/kg	10	73%
	1 cell/kg	10	22%
Heterogeneously contaminated	5 cells/kg in 1% of batch	10	< 2%
	104 cells/kg in 1% of batch	10	< 15%

¹ Assuming detection test is 100 percent effective (most methods are < 90 percent accurate).

Source: Mortimore and Wallace (1998).

In this example, with a contamination rate of *Salmonella* at 5 cells/kg and assuming the contamination is restricted to 1 percent of the batch, the probability of detecting the hazard by taking 10 samples of 25 g would be lower than 2 percent. If the contamination with *Salmonella* is homogeneously distributed at the same rate, the probability of detection would increase to 71 percent.

Table 5 shows that lot testing is not effective when defect rates are required to be low. In practice, a product safety defect rate of 1 percent would be absolutely intolerable in many food operations. Potentially, it represents 10 000 unsafe units per 1 million units manufactured. To detect this rate of contamination, however, 298 units would need to be sampled and tested to have 95 percent confidence that the contamination frequency was below 1 percent, and more than 459 samples must be tested and all found “negative” to be 99 percent confident.

It is evident that even the most elaborate sampling scheme and testing of end product cannot guarantee the safety of that product. There is no way to avoid some degree of error in acceptance or rejection of lots unless the entire lot is tested, in which case no edible food will be left.

2.2.1 Limitations of end-product testing – an example from the canning industry

A more illustrative example regarding the limitations of end-product sampling and testing is provided by the seafood canning industry (Ababouch, 2002). Canned seafoods are characterized by a pH > 4.6 and $a_w > 0.98$. Foods with a pH greater than 4.6 are called “low-acid canned foods” (LACFs), for which the micro-organism of major concern is *Clostridium botulinum* because of the deadly neurotoxin it can produce in foods. Some strains of *C. botulinum* produce spores that are the most heat resistant of all pathogenic micro-organisms. Consequently, the fish canning industry must rely on thermal processes sufficient to ensure the lowest possible probability of survival of *C. botulinum* spores so as to present no significant health risk to consumers. Experience has shown that the minimum heat process necessary to preserve an LACF should enable the reduction of the most heat resistant *C. botulinum* spores to 10^{-12} of its initial count. This is known as the botulinum cook or the 12D concept (Stumbo, 1973; Pflug, 1980), where D is the thermal reduction time or the time necessary to inactivate 90 percent of a given microbial population by heating at a constant temperature.

Stumbo (1973) reported that it is probably safe to assume that on the average, resistant *C. botulinum* spores contaminate foods at a rate of no more than one spore per container. Thus, a thermal process based on the 12D concept should achieve a probability of survival of one spore in one of one trillion containers. In other words, the probability for one container to be non-sterile is equal to 10^{-12} , i.e. one can in one trillion cans contains a viable spore of *C. botulinum*.

Because of this very low target probability of survival of *C. botulinum* spores in thermally preserved products, sampling and examining end products is not reliable to ensure product safety. Indeed, it is impossible to verify in a production lot that the probability for any one container to be non-sterile is < 10^{-12} . Table 7 shows that the

probability of finding at least one container that is not sterile in a random sample of size n is a function of n and of the percentage of non-sterile containers in the lot of processed containers.

TABLE 7

Probability of finding at least one container non-sterile in a sample of size n

Percentage of non-sterile containers in lot	Number of units in random sample						
	10	20	50	100	500	1000	10.000
0.001	0.000100	0.000200	0.000500	0.001000	0.004988	0.0098850	0.095164
0.002	0.000200	0.000400	0.001000	0.001998	0.009950	0.019802	0.181271
0.005	0.000500	0.001000	0.002497	0.004988	0.024691	0.048772	0.393477
0.01	0.001000	0.001998	0.004988	0.009951	0.048773	0.095167	0.632139
0.02	0.001998	0.003992	0.009951	0.019803	0.095172	0.181286	0.864692
0.05	0.004989	0.009953	0.024696	0.048782	0.221248	0.393545	0.993270
0.1	0.009955	0.019811	0.048794	0.095208	0.393621	0.632305	0.999955
0.2	0.018921	0.039249	0.095253	0.181433	0.632489	0.864935	1.000000
0.5	0.048890	0.095390	0.221687	0.394230	0.918428	0.993346	1.000000
1.0	0.095618	0.182093	0.394994	0.633968	0.993430	0.999957	1.000000
2.0	0.182927	0.332392	0.635830	0.867380	0.999959	1.000000	1.000000
5.0	0.401263	0.641514	0.923055	0.994079	1.000000	1.000000	1.000000
10.0	0.651322	0.878423	0.994846	0.999973	1.000000	1.000000	1.000000

Source: Pflug (1980).

For example, if this percentage is 0.01 percent (10^{-4}), the probability of finding one non-sterile container in a sample of 10 000 containers is only 0.63. This probability, based on a Poisson distribution, is very low (0.095) for a percentage of non-sterile containers equal to 0.001 percent (10^{-5}), and almost nil for a percentage equal to 0.0001 percent (10^{-6}) or less, not to mention that it is not feasible to draw and analyse a sample of 10 000 containers. In light of these data, it is legitimate to question the soundness of end-product sampling and analyses as requested by some food inspection authorities around the world. Not only it is not reliable, but most worrying is the fact that it could falsely infer commercial sterility (Ababouch, 2002).

2.3 SO WHAT WORKS?

Consequently, to ensure high levels of food safety and consumer protection, it is imperative to rely on an approach that prevents the hazard from entering the supply chain at the source or reduces its likelihood to acceptable levels, reflecting proper application of codes of practices, control and corrective measures.

While there is growing evidence that the implementation of HACCP-based systems have contributed to improving fish safety and quality, there has been a growing awareness of the importance of an integrated, multidisciplinary approach to safety and quality, considering the entire fish food chain. The food chain approach is recognition that the responsibility for the supply of food that is safe, healthy and nutritious is shared along the entire food chain – by all involved with the production, processing, trade and consumption of food.

In fisheries and aquaculture, there are five broadly defined needs on which a strategy in support of the food chain approach to food safety should be based:

- Fish safety and quality from a food chain perspective should incorporate the three fundamental components of **risk analysis** (assessment, management and communication) and, within this analysis process, there should be an **institutional separation** of science-based risk assessment from risk management – which is the regulation and control of risk.

- **Tracing techniques** (traceability) from the primary producer (including animal feed and medicines used during production), through post-harvest treatment, processing and distribution to the consumer must be improved.
- **Harmonization of fish quality and safety standards**, implying increased development and wider use of internationally agreed, scientifically based standards, is necessary.
- **Equivalence in food safety systems** – achieving similar levels of protection against fish-borne hazards and quality defects whatever means of control are used – must be further developed.
- Increased emphasis on **risk avoidance or prevention at source** within the whole food chain – from farm or sea to plate – including development and dissemination of good aquaculture practices (GAPs), GMPs and safety and quality assurance systems, i.e. HACCP, are necessary to complement the traditional approach to fish safety and quality management based on regulation and control.

The implementation of the food chain approach requires an enabling policy and regulatory environment at the national and international level with clearly defined rules and standards, establishment of appropriate food control systems and programmes at the national and local level, and provision of appropriate training and capacity building. Development and implementation of GAPs, GHPs and HACCP are required in the food chain steps. Government institutions should develop an enabling policy and a regulatory environment, organize the control services, train personnel, upgrade the control facilities and laboratories and develop national surveillance programmes for relevant hazards. The industry should upgrade facilities, train personnel and implement GAPs, GHPs and HACCP. The support institutions (academia, trade associations, private sector, etc.) should also train personnel involved in the food chain, conduct research on quality, safety and risk assessments, and provide technical support to stakeholders. Finally, consumers and consumer advocacy groups have a counterbalancing role to ensure that safety and quality are not undermined by political or economical considerations solely when drafting legislation or implementing safety and quality policies. They also have an important major role in educating and informing the consumer about the major safety and quality issues.

In many parts of the world, the food industry has adopted more elaborated safety and quality management systems that provide for better integration, coordination and traceability along the supply chain. Such schemes are voluntary and based on the generic quality schemes that have been developed under the aegis of the ISO for industrial products, e.g. Total Quality Management (TQM), ISO 9000:2000, ISO 22000 series. They are not discussed here and the interested reader should consult the relevant documentation.

For the fish industry, the proportion of the sector that has embraced these schemes and the additional quality and safety improvements they bring are not known. However, it is likely that most fish and seafood traded worldwide is produced with the view to meet the regulatory requirements of the destination markets. These requirements, and their concordance with international standards, codes and guidelines such as those of the CAC, are discussed in detail in Chapter 7.

The concept of risk analysis is detailed hereafter. The ensuing chapters analyse the various risks associated with fish and seafood and describe modern preventive approaches for consumer protection and the promotion of fair, responsible and transparent fish trade.

2.4 RISK ANALYSIS

Food-borne illnesses continue to be a major public health problem worldwide. It is estimated that up to 30 percent of the population in industrialized countries are

affected annually, and the situation in developing countries could be worse (WHO, 2007). Seafoods can also cause food-borne illnesses, including those due to the presence of “microbiological hazards”. The CAC has defined “hazard” as a biological, chemical or physical agent in, or condition of, food with a potential to cause an adverse health effect. There are many hazards but not all have the same severity (e.g. low levels of histamine in fish, while relatively common, do not always result in illness in the consumer, whereas botulinum toxin, while rare, often causes death or severe illness with long-term sequelae). The public health significance of seafood-borne illnesses depends on the probability of illness (number of cases) and the severity of illness. For prioritization of food safety management activities and appropriate allocation of resources, there is a need for a way to compare the “importance” of different food-borne hazards, where importance is usually related to public health affect.

Food safety risk has been defined by the CAC (2011) as a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard in food. As a consequence of agreements made internationally through the completion of the Uruguay Round of the General Agreement on Tariffs and Trade (GATT) in 1994, the idea of “risk analysis” has become the method for establishing tolerable levels of hazards in foods in international trade and, equally, within national jurisdictions. Risk analysis may be performed at the national level or at the international level by the CAC. Food safety issues may be brought to the CAC by member countries.

The objective of the rules that govern international trade in food, the SPS and TBT Agreements (WTO, 2010), is to ensure equitability in international trade in foods and to permit countries to set food safety management measures for their populations and ask that imported foods afford the same level of public health protection. To justify and compare the levels of public health protection and related food safety measures, risk sources must be analysed, the risk estimated and risk management options evaluated using the risk analysis framework described by the CAC (1999).

“Risk analysis” is the name given to the process now underlying the development of food safety standards (FAO/WHO, 1997). It consists of three separate but integrated parts:

- risk management;
- risk assessment;
- risk communication.

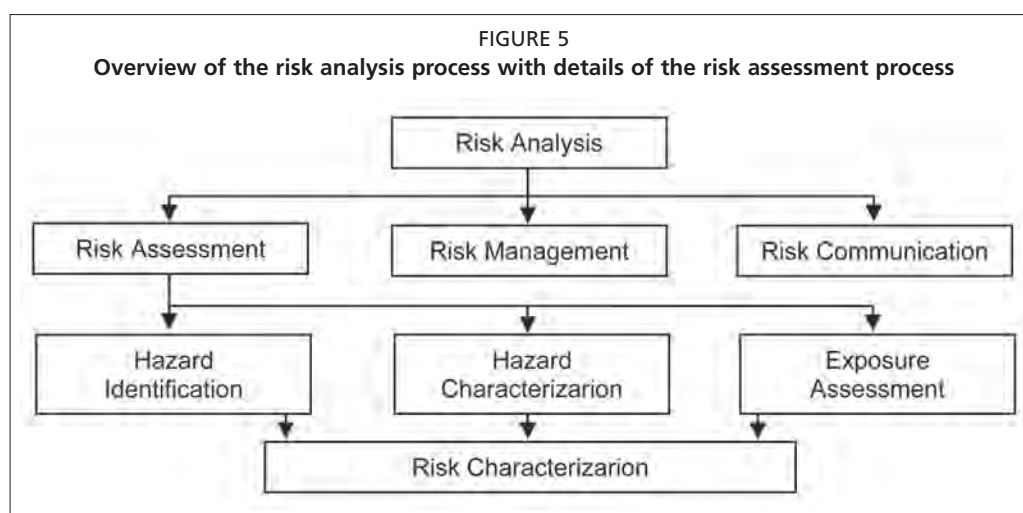
The management and control of seafood-borne diseases is carried out by several groups of people. It involves technical experts assessing the risk, i.e. synthesizing epidemiological, microbiological and technological data about the pathogenic agent, the food, the host, etc. It involves both risk managers at the government level, who have to decide what level of risk society will tolerate, i.e. while balancing other considerations (e.g. the cost of risk management measures, and their affect on the affordability and utility of foods) and risk managers in both industry and government that have to implement procedures to control the risk to satisfy those societal expectations. In the current international food safety management environment, those expectations are expressed as “food safety objectives” (FSOs), which are translated into practical targets and advice for industry as “performance objectives” (POs) and “process criteria” (see Chapter 6). At industry level, these objectives and criteria are satisfied using GHP and HACCP procedures, as described above (also see Chapter 6).

The risk analysis process must be “transparent”, that is, clearly and fully articulated; and at every step, all stakeholders should be allowed to participate and comment. It has been seen as important that there is a separation between the processes of risk management and risk assessment (FAO/WHO, 1995) in order to avoid bias leading to desired outcomes in the risk assessment process. The CAC recommends functional separation of risk assessment and risk management to ensure the scientific integrity of risk assessment and to avoid confusion over the functions to be performed by risk

assessors and risk managers and reduce conflict of interest. Risk assessment is required to be an objective, “science-based”, evaluation of risk.

The three components of risk analysis and their interrelationships are shown in Figure 5, and they have been defined by the CAC. Those components are:

- **Risk assessment:** A scientifically based process consisting of four steps described as: (i) hazard identification; (ii) hazard characterization; (iii) exposure assessment; and (iv) risk characterization.
- **Risk management:** The process, distinct from risk assessment, of weighing policy alternatives in consultation with all interested parties (“stakeholders”), considering risk assessment and other factors relevant for the protection of health of consumers and for the promotion of fair trade, and, if needed, selecting and implementing appropriate prevention and control options.
- **Risk communication:** The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.



2.4.1 Risk management

When a food safety issue is brought to the attention of risk managers, they should initiate the process of risk analysis. The food safety issue may arise owing to consumer concern or through epidemiological data, surveillance or through concern raised by a trade partner. FAO/WHO (1997) elaborated the general principles of risk management:

- Risk management should follow a structured approach.
- Protection of human health should be the primary consideration in risk management decisions.
- Risk management decisions and practices should be transparent.
- Determination of risk assessment policy should be included as a specific component of risk management.
- Risk management should ensure the scientific integrity of the risk assessment process by maintaining the functional separation of risk management and risk assessment.
- Risk management decisions should take into account uncertainty in the output of risk assessment.
- Risk management should include clear interactive communication with consumers and other interested parties in all aspects of the process.

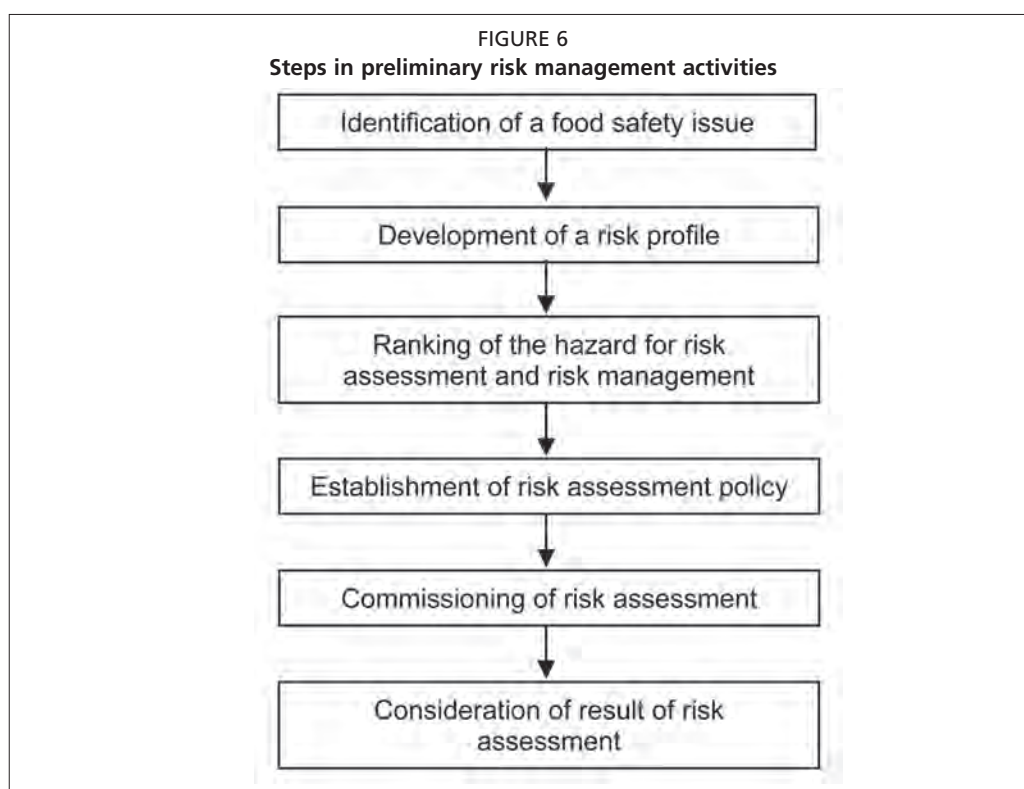
- Risk management should be a continuing process that takes into account all newly generated data in the evaluation and review of risk management decisions.

The structured approach for risk management described in FAO/WHO (1997) includes four major steps:

1. preliminary risk management activities;
2. identification and selection of risk management options;
3. implementation of risk management decision;
4. monitoring and review;

Preliminary risk management activities (CAC, 2007a) consist of steps illustrated in Figure 6. Food safety issue needs to be identified by the risk manager in collaboration with interested parties.

The recognition of methylmercury as a food-borne hazard in the 1950s, following an outbreak of severe neurological disease in babies of mothers who ate fish from Minamata Bay in Japan, is an example of **identification of a food safety issue** (FAO/WHO, 2006a). Recognition of *Listeria monocytogenes* as a food-borne pathogen after a 1981 outbreak of listeriosis in Canada, traced to contaminated coleslaw (Swaminathan *et al.*, 2007), is another example. Risk managers may need to take immediate action when public health concern demands urgent response, such as the discovery of acrylamide in certain foods in 2001 (WHO, 2002), but such measures should be temporary, subject to review within a time frame, and clearly communicated.



A **risk profile** is undertaken to provide a concise description of the current state of knowledge related to the food safety problem, potential risk management options and food safety policy context that would influence risk management actions. The risk profile also tries to evaluate whether there is sufficient cause for concern that a risk assessment should be undertaken and also whether there is sufficient data to complete a risk assessment to answer relevant risk management questions. Examples

of risk profiles relevant to seafoods include those prepared by the New Zealand Food Safety Authority concerning *Vibrio parahaemolyticus* (Lake, Hudson and Cressey, 2003); ciguatoxin (Cressey, Gilbert and Lake, 2007) and norovirus in raw molluscs (Greening *et al.*, 2009). Typical risk profiles include a brief description of:

- the real or perceived problem;
- the food product or commodity involved;
- pathways by which consumers are exposed to the hazard in the food;
- effect of processing steps on the level and frequencies of presence of the hazard;
- potential consequences of human exposure to the hazards (i.e. types and severity of illness caused);
- factors affecting host susceptibility and including whether probability of exposure or severity of consequences differs among different segments of population;
- consumer perception of the risks.

In some cases, a risk profile will provide sufficient information to identify and select appropriate risk management actions, or reveal that there is insufficient risk for any action to be needed. In other situations, the risk profile will result in articulation of actions needed to better understand and manage the risk (e.g. gathering more data to better resolve the risk), and/or the commissioning of a risk assessment. As part of the decision to undertake a risk assessment, the questions to be addressed by the risk assessment need to be clearly articulated by the risk manager, and the resources – and time – available to complete the risk assessment also need to be made clear. While the task of risk assessment should be done independently of decisions about risk management, experience has shown that there needs to be significant interaction between risk assessors and risk managers so that the risk assessment proceeds efficiently and responds to the risk managers' needs for support for the decisions that they need to make. In addition, dialogue between the risk managers and the food industry and consumers is necessary for making decisions that are technologically achievable and also satisfy societal concerns and expectations of food safety.

Developing regulatory standards, microbiological specifications or other risk management measures may require a risk assessment to be performed. While deciding whether to proceed with a formal risk assessment, risk managers need to consult with risk assessors to consider how a risk assessment could be approached, what questions could be answered and whether data gaps and uncertainties could preclude unequivocal answers. Identification of key data gaps would facilitate collection of additional data before and during risk assessment, and this might require the involvement of government departments, academic institutions and the food processing industry.

“Risk assessment policy” has been defined by the CAC (2011) as “documented guidelines on choice of options and associated judgements for their application at appropriate decision points in risk assessment such that scientific integrity of the process is maintained”. Establishment of risk assessment policy by risk managers should be carried out in consultation with risk assessors and other stakeholders. Documentation of that policy is necessary to ensure consistency, clarity and transparency. Risk assessments require resources – both scientific expertise and also financial resources. Risk managers assemble a team of experts to carry out the task, and the team needs to have expertise in relevant disciplines such as public health, epidemiology, food microbiology, toxicology, food technology, biostatistics and modelling to be able to fully assess the risk and the importance of various risk-affecting factors.

2.4.2 Risk assessment

Risk assessment is undertaken to provide support for decisions that confront risk managers. As such, risk assessors should present the outputs of risk assessments in such a way that the risk managers are fully informed of the strengths and limitations of those

risk estimates, as well as the suitability of the various risk management options to deal with the food safety issue. When risk managers have to deal with several food safety issues at a given time, risk assessments may help rank these issues based on the relative risk to health from each of those hazards, and set priorities for risk management. A risk assessment in the United States of America (FDA/USDA/CDC, 2003) was used to rank the relative risk to consumers from *L. monocytogenes* in 23 categories of ready-to-eat (RTE) foods. From that ranking, priorities for risk management were decided. In general, the risk that each issue presents to the consumer is the primary consideration for ranking, but other considerations such as impact of any proposed control measures on trade may also be considered by risk managers. A holistic approach to risk analysis is required so that the benefits of risk management actions outweigh any increased costs to consumers, e.g. increased cost of food, loss of utility and loss of choice. This holistic approach should also extend to the risk assessment.

As mentioned above, food safety risk assessment involves four steps. These steps are further elaborated below.

Hazard identification involves collation and analysis of epidemiological data and evidence for the link between the food, the hazard and human illness. Data could be from national surveillance studies, outbreak investigations, clinical studies and food process evaluations. In the case of pathogens, the organism, its characteristics, pathogenicity and factors involved in causing human illness (e.g. toxins, adhesins, etc. either pre-formed in food before consumption or produced by the organism after infecting the host) and symptoms of illness are also documented.

Hazard characterization is a qualitative or quantitative description of the severity and the duration of the adverse health effects that may result from the ingestion of the micro-organism or toxin. The virulence characters of the pathogen, the effect of food matrix on the organism at the time of consumption (e.g. high fat content in a food may protect the organism against gastric acidity and increase its chances of surviving passage through the gut to the intestine where it may establish infection), host susceptibility factors and population characteristics are considered. Wherever data are available, a dose-response analysis is performed that aims to quantify the probability and/or severity of illness in consumers as a function of the dose of toxin, or pathogens, that are ingested. Data for dose-response analysis may come from outbreak investigations, human volunteer studies, vaccine trial studies, animal studies, etc. In general, models for the dose-response relationship of pathogens are actually models that relate the dose ingested to the **probability** that an infection will ensue. The models that are used assume that increasing doses of the pathogen increase the probability of infection in simple proportion – up to some upper dose beyond which no further risk increase occurs.

Exposure assessment is concerned with estimating the likelihood that consumers will be exposed to the hazard through consumption of the food under consideration, and also the dose to which an individual or population is exposed. In microbial food safety risk assessment, for example, an estimate of frequency of exposure to the hazard in the food is developed, together with an estimate of the numbers of the pathogen or the level of a biotoxin consumed via the food (or foods) of concern. This involves documenting the sources, frequency and levels of contamination and factors that alter the concentration of frequency of the hazard between harvest and consumption, e.g. processing steps that remove, dilute or kill/denature the hazard, or alternative time-temperature conditions that permit pathogen growth.

Microbial hazards in foods are much more dynamic than chemical hazards because of the potential of micro-organisms to multiply in foods or for their numbers to be reduced by inactivation processes. With respect to microbial toxins, a combination of the characteristics of microbe and the physiological effects of the toxin, the stability of the toxin, the conditions under which the toxin is synthesized, etc. need be considered.

Data on the concentration of the pathogen in the food at the time of consumption are rarely available and, therefore, it is necessary to develop models or assumptions to estimate the likely exposure. For bacteria, the growth and death of the organism under the predicted handling and processing conditions of the food are considered in the model, which must take into account the effects on the pathogen related to time, temperature, food chemistry and the presence of competing microflora. Biological agents such as viruses and parasites do not multiply in food handling. However, storage and processing conditions may affect their survival.

Accordingly, knowledge of the microbiology ecology of pathogens in foods is an essential component of exposure assessment to be able to predict the effects of product formulation, time and storage temperature and, increasingly, mixtures of gases in the product storage environment (or retail package) on pathogen growth, death or survival. Such knowledge includes the quantitative effect of temperature, pH, presence of antimicrobial agents such as organic acids (or their salts), essential oils (more common in traditional foods), competing microbiota, etc. Knowledge of consumer handling practices (e.g. home refrigeration temperatures and cooking practices) is also important. The subject of microbial ecology in risk assessment was discussed by Ross (2008). As well as the concentration and frequency of pathogens in foods, exposure depends on the amount eaten both in terms of frequency of eating the food of interest and the size of the serving. Thus, knowledge of population demographics, food serving size, food preparation practices and consumption patterns for different groups within the exposed population is also part of an exposure assessment.

The CAC defines the **risk characterization** step as the process of qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and the severity of the known or potential adverse health effect in a given population based on hazard identification, exposure assessment and hazard characterization.

Thus, in the risk characterization step, the information generated and collated in hazard identification, exposure assessment and hazard characterization is collated and analysed to produce an understanding of factors that affect the risk from the food-borne hazard being considered and to produce estimates of the attendant risk. Ideally, the risk characterization should provide insights into the nature of the risk, how it arises and the uncertainty in the risk estimate, e.g. identification of the most important factors contributing to the risk, sources and influence of uncertainty and variability in the risk estimate, and identification of gaps in data and knowledge and the influence of those gaps on the confidence one can have in the risk estimate. Often, the risk characterization includes consideration of how the risk might change under different scenarios, e.g. to explore the effectiveness of different risk management options that could be adopted.

The risk estimate may be:

- qualitative (e.g. using descriptive, but often subjective, terms such as “low”, “medium”, “high”);
- semi-quantitative, in which the level of risk is compared with some other risk, or in which the risks from different sources are put in order of severity (the ranking itself may be quantitative although not calibrated to absolute burdens of disease);
- quantitative (the risk assessors predicting the risk per meal serving or the number of people in a defined population who are likely to become ill from the pathogen–commodity/product combination).

Guidelines for the conduct of microbial food safety risk assessment have been developed by the CAC (1999).

A qualitative risk assessment may be performed where data are inadequate to make numerical estimates but where prior expert knowledge and identification of attendant

uncertainties are sufficient to permit risk ranking or separation into descriptive categories of risk. For example, Huss, Jørgensen and Fonnesbech Vogel (2000) estimated risk from consumption of molluscan shellfish, fish eaten raw, lightly preserved fish and mildly heat-treated fish as “high”. Seafood products estimated to cause “low risk” to consumers included chilled/frozen fish and crustaceans, semi-preserved fish and heat-processed (canned) fish. The risks from dried and heavily salted fish were considered to come from pathogenic bacteria. Sumner and Ross (2002) presented a qualitative risk assessment for methylmercury in seafood.

“Risk Ranger”, developed and described by Ross and Sumner (2002), is an automated Microsoft® Excel® spreadsheet, and is an example of a semi-quantitative risk assessment tool. It requires the user to choose from a range of answers or to enter data in response to questions related to the following risk criteria: (i) hazard severity; (ii) relative susceptibility of the population exposed to the hazard; (iii) frequency of consumption; (iv) proportion of the population consuming the food of interest; (v) size of the population being considered; (vi) probability of contamination of the food by the hazard; (vii) the effect of the process on hazard levels; (viii) the possibility of recontamination after processing; (ix) the effectiveness of post-process controls and handling to prevent pathogen/toxin increase; (x) the increase in the initial pathogen load that would be required to lead to an “infective dose”; and (xi) effect of treatment (e.g. cooking) prior to consumption on the levels of the hazard ingested. The outputs of this risk assessment tool include a relative ranking of the risk (on a scale of from 0 to 100) or an estimate of the number of illnesses in the population of interest per year. Sumner, Ross and Ababouch (2004) described application of Risk Ranger to estimate the risk of ciguatera fish poisoning in New Zealand.

Quantitative risk assessments can be categorized as deterministic or probabilistic (or “stochastic”). For deterministic risk assessment, single input values that best represent each of the risk-affecting factors in the system being considered are chosen. The values could represent the most likely value or values that represent the “worst case”. Deterministic risk assessment does not provide information on the uncertainty of the risk estimate, or on the range of risk under all sets of realistic circumstances. Moreover, selecting and combining worst-case input values for multiple factors affecting food safety (e.g. highest storage temperature, worst contamination level, longest storage time, and most virulent strain) may lead to overly conservative estimates of the risk to consumers and, in turn, to unnecessarily stringent risk management actions. In the case of probabilistic (stochastic) risk assessments, input values are distributions of possible values including some characterization of upper and lower extremes and the most “usual” value, or situation. These distributions can represent “real world” variability and/or uncertainty in input values. Stochastic risk assessment is usually undertaken using computer simulation software. It has the advantage of providing a full representation of the risk estimate (which is itself uncertain) including the average value of the estimated risk, the estimate of the most likely, as well as risk estimates that correspond to different levels of confidence (e.g. the risk estimate that encompasses 95 percent of the situations, or “scenarios”, predicted by the risk model). Uncertainty analysis is a method used to estimate the uncertainty associated with models and assumptions used in the risk assessment.

From experience to date, microbial food safety risk assessments often conclude that insufficient data were available in one or more areas and, as a result, there is uncertainty about the true level and/or range of risk, as expressed in the risk estimate. It is important to record the data that are not available (i.e. the “data gaps”) that lead to that uncertainty. Later, if that knowledge becomes available, the level of uncertainty will be reduced so that the risk estimate becomes more accurate. Risk assessment is an iterative process and may need re-evaluation as new data become available. Wherever possible, risk estimates should be reassessed over time by comparison with independent human

illness data. Two examples of risk assessments relevant to seafood undertaken by WHO/FAO are: (i) a risk assessment of *Listeria monocytogenes* in RTE foods; and (ii) a risk assessment of *Vibrio parahaemolyticus* in seafood.

2.4.3 Translating risk estimates into risk management – food safety objectives

When determining a public health goal, risk is most often expressed as a number of cases of illness per capita per year. For instance, the baseline level of listeriosis cases in the United States of America in 1997 was 0.5 per 100 000 of the population per year. The White House announced in the “Healthy People 2010” programme that this should be reduced to 0.25 cases per 100 000 of the population per year; that is, the United States Government set a food safety objective.

Several terms exist for such public health goals. Ideally, the goal would be to reduce all seafood-borne diseases to “zero risk”. However, this is technically and financially not possible. It is important to understand that there is no such thing as “absence of risk”. Therefore, the public health goal is often expressed using terms such as “appropriate level of protection” (ALOP).

Levels of disease attack rate are difficult to measure and target by food safety managers in government and industry and therefore the term “food safety objective” (FSO) has been introduced. The FSO translates risk into a measurable goal, and this is expressed as the concentration or frequency of a hazard in a food (at the point of consumption) that is considered “safe” or that meets the level of protection/risk set by society. While FSO has been used in broad terms by several authors (Jouve, 1996; Hathaway, 1997), it has been explicitly defined by the International Commission on Microbiological Specifications for Foods (ICMSF) (van Schothorst, 1998; ICMSF, 2005).

Food safety objective

The maximum frequency or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ICMSF, 2005).

If a quantitative risk assessment has been conducted, the FSO can simply be the translation for the Y-axis on a plot of risk estimate versus cumulative probability (effectively the degree of confidence that one can have that the risk is below a specified level) to the X-axis (with the number or frequency of the pathogen or some other measure of “risk”).

Even where quantitative risk assessments and the risk characterization curve are not available, FSOs are still often set. Investigations of food-borne diseases, epidemiological surveillance programmes, industry records and knowledge of the influence of food processing parameters have for decades provided information about which pathogens most often cause food-borne illness, which foods are implicated, sometimes the levels of pathogens that are involved, and other factors that have contributed to the hazard being realized in terms of human food-borne illness (e.g. poor hygiene, temperature abuse, and inadequate processing). In effect, the setting of microbiological criteria for foods has been and is an indirect way of setting an FSO – and thus implies a desired public health goal. Many examples of this exist. One is the standard for *Staphylococcus aureus* in cooked crustaceans ($n = 5$, $c = 2$, $m = 100/\text{g}$ and $M = 1\,000/\text{g}$). This criterion implicitly contains an evaluation of the risk related to the concentration of the hazard (growth and high concentrations are required to produce the amount of enterotoxin causing disease) (ICMSF, 1986). Often, however, the connection between risk and microbiological criteria is, at best, obscure.

It is important to realize that FSOs are not equivalent to microbiological criteria but that, if appropriate, criteria can be derived from FSOs. An FSO is a public health goal whereas a microbiological criterion defines acceptability of a food product or a lot of foods and should indicate the sampling plan, method, number of units that must conform, etc. An example of an FSO is a concentration of 100 *L. monocytogenes* per gram at the point of consumption for RTE foods (EC, 2005a, 2007a; CAC, 2007b, 2009a). Criteria for *L. monocytogenes* at earlier points in the chain will typically be lower than the 100 cfu/g and the concept of a “performance objective” (PO) has been coined to translate the FSO into a target level or frequency of contamination that industry can aim for, at the point of production, so as to ensure that the FSO is consistently met. The FSO is a target for the food chain to reach, but it does not specify how the target is to be achieved. Hence, the FSO offers flexibility to the food chain to use different operations and processing techniques that best suit the situation, provided that the maximum hazard level specified at consumption is not exceeded. In products such as RTE foods, the POs can be calculated from the FSO by subtracting expected bacterial contamination and/or growth between the point of manufacture/processing and the point of consumption, taking into account potential changes in microbial levels and frequencies of contamination, e.g. due to growth, inactivation processes, cross-contamination and dilution.

An authority can use FSOs and POs to communicate appropriate food safety levels to industry and other governments. The FSOs and POs are levels of food-borne hazards that should not be exceeded at the point of consumption and earlier in the food chain, respectively. They can be met using good practices (GAPs and GHPs) and HACCP programmes (ICMSF, 2005).

It must also be determined whether the FSO, as expressed by risk managers, is achievable using existing industry practices and technologies. If not, it is necessary to decide whether: (i) changes in the industry have to be enforced; (ii) the product should be taken off the market; or (iii) the product should be labelled as carrying a risk. Examples of such procedures are: (i) the mandatory pasteurization of milk; (ii) the banning of fish species containing tetrodotoxin for the market of the European Union; and (iii) the notice by restaurants in several states in the United States of America that eating raw oysters may be detrimental to health. Examples of FSOs are shown in Chapter 6.

2.4.4 Risk communication

Risk communication is an integral part of risk analysis. It provides timely, relevant and accurate information both between the members of the risk analysis team and external stakeholders. Internal risk communication should take place between different groups in risk analysis, i.e. risk assessors, risk managers and risk communicators. External risk communication deals with information exchange between the risk analysis team and external stakeholders (FAO/WHO, 1999). Where stakeholders are asked to review and comment on the risk assessment and are consulted about potential risk management options, they are more likely to accept the risk management approaches proposed. Equally, if the risk manager has a good understanding of how the risk affects and, importantly, is perceived by stakeholders (see below), and the willingness and capacity of stakeholders to manage some aspects of the risk themselves, better risk management decisions are likely to result. For example, stakeholders will be more willing to tolerate some risk if they perceive that the risk also offers some benefit to them.

Stakeholder perception of risk has both technical and emotional dimensions, and risk communication should address both these aspects. Often, non-technical information emphasized by media, consumer groups or industry captures the attention of the general public that are exposed to the risk. Risk communication should address the concerns of the public and not dismiss these as irrational. FAO/WHO (2005a)

recommended that risk communication should pay attention to the following: (i) collection and analysis of background information about food safety risk, the context and perception of different stakeholders; (ii) developing and disseminating key messages targeted at particular audiences; (iii) engaging stakeholders in dialogue about the risk; and (iv) monitoring and evaluating the outcomes of risk communication.

FAO/WHO (2005a) identified the following as necessary components of effective risk communication:

- the nature of the risk – including its magnitude and severity, nature and size of the population at risk, probability of exposure and amount of exposure that constitutes a significant risk (N.B.: these are the elements of hazard identification);
- the risk assessment itself – including the methods used, weaknesses or inaccuracies in the available data, assumptions on which the estimate is based, uncertainties, sensitivity of the estimate to changes in the assumptions, and effect of changes in the estimates on risk management decisions;
- the risk management decisions proposed – including explanation of the reasons for choosing a particular option, its likely effectiveness, trade-off between risks and benefits, costs of managing the risks and who pays for the cost of managing the risk.

As stakeholder groups can be expected to be heterogeneous, e.g. including primary producers, industry, distributors and vendors, consumer groups, and the general public, risk communicators need to understand their audience, and they may need to involve experts to help to articulate credible messages, to assure transparency, to put the risk in the right perspective, to differentiate between scientific and value judgement, etc.

3. Characterization of hazards in seafoods

3.1 THE DISEASE BURDEN DUE TO SEAFOOD (IDDYA KARUNASAGAR)

The global burden of food-borne disease is unknown, and this is mainly because of the lack of obligation to report the illnesses to public health authorities. Many individuals affected by food-borne illnesses may not seek medical care, and the causative agents may not be identified by appropriate laboratory investigations. However, some countries have surveillance programmes, and the results of these give an indication of the disease burden. Although the data are mostly from developed countries, this fact should be considered against the background that underreporting occurs even in these countries and, most often, the incriminated food is not available for analysis and the aetiological agent is not identified.

Estimates in the United States of America indicate that, annually, 48 million food-borne illnesses involving 128 000 hospitalizations and 3 000 deaths occur (Gillis *et al.*, 2011). Pathogens are incriminated in 9.4 million cases involving 55 961 hospitalizations and 1 351 deaths (Scallan *et al.*, 2011a). Unspecified agents are involved in an estimated 38.4 million cases leading to 71 878 hospitalizations and 1 686 deaths (Scallan *et al.*, 2011b). Norovirus accounts for a large proportion of these illnesses (58 percent) followed by non-typhoidal *Salmonella* (11 percent), *Clostridium perfringens* (10 percent), and *Campylobacter* (9 percent). There are few studies in which the foods incriminated have been specified. Data from the Centers for Disease Control and Prevention (CDCs) show that in the period 1993–97 there were 2 751 food-borne outbreaks involving 86 000 people and 29 deaths and that in only one-third of the outbreaks were the foods implicated identified. Fish and shellfish accounted for 187 outbreaks with 2 564 people affected and no deaths (Olsen *et al.*, 2000). In the period 1998–2002, 6 647 outbreaks were reported involving 128 370 people and 88 deaths (Table 8).

TABLE 8
Foods involved in food-borne illness in the United States of America, 1998–2002

Food	Outbreaks		Cases		Deaths	
	Number	Percentage	Number	Percentage	Number	Percentage
Beef	208	3.1	4 189	3.3	5	5.6
Dairy	92	1.4	2 231	1.7	0	0
Eggs	83	1.2	2 212	1.7	0	0
Game	10	0.1	91	0.0007	0	0
Pork	138	2.1	2 699	2.1	0	0
Poultry	345	5.2	4 987	3.9	15	17.0
Vegetables	192	2.9	7 037	5.5	4	4.5
Fruits and nuts	87	1.3	3 496	2.7	3	3.4
Grains	81	1.2	1 148	0.9	0	0
Oils and sugars	12	0.2	265	0.2	0	0
Finfish	337	5.1	1 692	1.3	1	1.1
Shellfish	151	2.3	1 758	1.4	0	0
Unclassifiable vehicle	232	3.5	5 335	4.2	3	3.4
Complex vehicle	2 079	31.3	45 046	35.1	39	44.3
Known vehicle	4 047	60.9	82 186	64.0	70	79.5
Unknown vehicle	2 600	39.1	46 184	36.0	18	20.5
Total	6 647	100.0	128 370	100.0	88	100.0

Source: Lynch *et al.* (2006).

The vehicle of transmission was unknown in 39 percent of outbreaks, 36 percent of cases and 20.5 percent of deaths. “Complex vehicle” was responsible for 31 percent of outbreaks, 35 percent of cases and 44 percent of deaths. The aetiology could be confirmed in 53.7 percent of cases, including 29.5 percent of bacterial, 22 percent of viral, 0.6 percent of biotoxins, 0.5 percent of parasites cases (Lynch *et al.*, 2006). Fish and shellfish accounted for 488 outbreaks (7.3 percent) involving 3 450 cases (2.7 percent) in the period 1998–2002. Ciguatoxin and scombrototoxin accounted for the highest number of outbreaks (Table 9). Among bacteria, *V. parahaemolyticus* was a major aetiological agent. Other bacterial agents involved included *Clostridium botulinum*, *Salmonella*, *Vibrio cholerae*, *Bacillus cereus*, *Shigella* and *Campylobacter* (Table 9).

Using data from CDC and state health departments, DeWaal *et al.* (2006) estimated that in the period 1990–2003, there were 4 486 outbreaks involving 138 622 cases, of which 899 outbreaks (20 percent) involving 9 312 persons (7 percent) were due to seafood. Scombrototoxin accounted for 38 percent (341 out of 899) and ciguatoxin for 24 percent (215 out of 899) of outbreaks. Five hundred and seventy-one outbreaks involving 2 991 cases were due to finfish, such as tuna and grouper, 135 outbreaks (3 156 cases) were due to molluscan shellfish, 129 outbreaks (2 400 cases) were linked to crab cakes and sushi, and 64 outbreaks (765 cases) were due to other seafood such as shrimp and lobster (DeWaal *et al.*, 2006). The CSPI Outbreak Alert (CSPI, 2007) shows that, in the period 1990–2005, there were 1 053 outbreaks involving 10 415 cases associated with seafood in the United States of America. Scombrototoxin accounted for 36 percent of these, ciguatoxin, 22 percent, *Vibrio* and norovirus, 9 percent each, and “other bacteria” 16 percent. Tuna and grouper were involved in the largest number of outbreaks (661) (Table 10) but molluscan shellfish associated illness affected the largest number of people (3 535) in the seafood category. Crustaceans such as shrimp and lobster were in the smallest group. In the period 1999–2008, seafood accounted for 792 outbreaks involving 6 337 cases (DeWaal, Roberts and Catella, 2012).

TABLE 9

Aetiology of food-borne outbreaks associated with finfish and shellfish in the United States of America, 1998–2002

Aetiology	1998		1999		2000		2001		2002	
	Finfish	Shellfish	Finfish	Shellfish	Finfish	Shellfish	Finfish	Shellfish	Finfish	Shellfish
Bacterial										
<i>Bacillus cereus</i>		1			1		1			
<i>Campylobacter</i>		1								
<i>Clostridium botulinum</i>	2								2	
<i>Salmonella</i>	1	1			2		1		1	
<i>Shigella</i>	1						2			
<i>Vibrio cholerae</i>		1		1			1			
<i>Vibrio parahaemolyticus</i>		11		3		3		1		2
<i>Vibrio spp.</i>		1								
Total	4	16		4	3	3	1	5	3	2
Toxins and chemical										
Ciguatoxin	16		12		12		24		20	
Scombrototoxin	27		20		20		29		21	
Shellfish toxin		1				3			1	
Other chemical	1									
Total	44	1	32		32	3	53		42	
Viruses										
Hepatitis A		1				1				
Norovirus				2	1	2	1	8		2
Total		1		2	1	3	1	8		2
Parasitic			1							
Unknown aetiology	21	20	31	22	27	16	20	20	21	23
Total	69	38	64	28	63	25	75	33	66	27

Source: Lynch *et al.* (2006).

The results of surveillance for European countries are available from the website of the WHO Regional Office for Europe. The eighth report for the period 1999–2000 is available for individual countries, but the seventh report for the period 1993–98 has a summary for the continent. In 40 countries in the period 1993–98, there were 33 307 food-borne outbreaks involving 288 923 cases. A causative agent was identified for 70 percent (23 538) of the outbreaks. *Salmonella* accounted for 77 percent of outbreaks and other bacterial agents for 14.5 percent of outbreaks. Viruses accounted for only 1 percent of outbreaks. Fish and fishery products were involved in 1 208 out of 22 386 outbreaks (5.3 percent) in which the food involved was identified. Among foods involved in *Salmonella* outbreaks, fish and fishery products accounted for 1 percent. *Clostridium botulinum* was the causative agent in 67 outbreaks, of which 7 (10.5 percent) involving 83 cases were related to home-prepared fishery products.

TABLE 10

Seafood groups involved in food-borne outbreaks in the United States of America, 1990–2005

Seafood group	Outbreaks	Cases
Tuna, grouper	661	3 344
Molluscan shellfish	165	3 535
Seafood dish (e.g. crab cake, tuna burger)	157	2 658
Other seafood (including shrimp, lobster)	70	878
Total	1 053	10 415

Source: CSPI (2007).

In England and Wales, the United Kingdom of Great Britain and Northern Ireland, 1.7 million cases of food-borne illness occurred in the period 1996–2000, of which 116 603 (7 percent) were linked to seafood with shellfish accounting for 77 019 cases (4 percent) (Adak *et al.*, 2005). The disease risk score (cases/1 million servings) for seafood was 41, with shellfish having a high disease risk score (646). In the period 1999–2000, fish and shellfish were involved in about 18 percent of food-borne outbreaks in France.

In Australia, 5.4 million cases of food-borne gastroenteritis occur annually, and data on causative agents are available, but not on the incriminated foods (Hall *et al.*, 2005). The OzFoodNet Working Group (2006) report shows that, in Australia, there were 115 food-borne disease outbreaks that affected 1 522 persons. Fish were involved in 13 of these outbreaks (11 percent), and oysters in one outbreak. Seven outbreaks were due to ciguatera poisoning and four to histamine poisoning. Wang *et al.* (2007) noted that, between 1994 and 2005, a bacterial cause was identified in 1 082 food-borne outbreaks involving 57 612 cases and 51 deaths in China. *Vibrio parahaemolyticus* topped the list accounting for 19.5 percent of outbreaks and 18.7 percent of cases, followed by *Salmonella* (16.7 percent of outbreaks, 22.3 percent of cases). *C. botulinum* was involved in 2.8 percent of outbreaks, 0.4 percent of cases, but 62.8 percent of deaths. In Seoul, the Republic of Korea, 147 food-borne outbreaks involving 7 203 cases occurred in the period 2002–06, with bacterial agents being responsible for 42.6 percent and viral agents for 41.9 percent of cases (Lee *et al.*, 2009).

These data indicate the serious nature of food-borne illnesses from consumption of fish and other seafood, especially with regard to biological hazards. The following sections elaborate on these hazards, as well as on chemical and physical hazards associated with fish and fish products.

3.2 BIOLOGICAL HAZARDS

3.2.1 Pathogenic bacteria (Iddya Karunasagar and Tom Ross)

Bacteria that may cause illness in humans are considered pathogenic bacteria. This section discusses bacteria that are associated with illnesses following consumption of fish and fishery products. When fish are alive, bacteria are associated with their

surface, in the gills and in gut. Most bacteria associated with fish are not pathogenic. A few of them may be associated with spoilage and a few may be even involved in the production of certain fermented fish. Bacterial food-borne illnesses may be of two major types: food-borne infections and intoxications (Table 11). **Food-borne infections** are caused by the ingestion of live pathogenic micro-organisms (the minimum infective dose varies considerably among bacterial species) with the food, while **food-borne intoxications** are caused by ingestion of toxins produced by the micro-organisms in food. Most often, toxin-producing bacteria would have grown to high numbers (10^5 – 10^8 cfu/g) before the food is consumed. Intoxications might occur even when viable micro-organisms that have produced the toxin are no longer present in the food at the time of consumption, e.g. *Staphylococcus* produces heat-stable toxins and, therefore, the toxins can persist in heat-treated foods even after the bacteria are inactivated. There is another intermediary category of bacterial food-borne illness in which clinical symptoms are produced by the toxin produced by the micro-organism in the human system following infection (toxi-infection).

The outcome of ingesting food containing pathogenic bacteria depends on the level of pathogen and the food matrix. In general, pathogens such as *Shigella* and enterohaemorrhagic *Escherichia coli* (EHEC) have a low infective dose (ICMSF, 1996; Meng *et al.*, 2007). According to human volunteer studies, the infective dose of non-typhoidal *Salmonella* is generally about 10^6 cells (FAO/WHO, 2002), but in certain fatty foods (e.g. chocolates) that protect pathogenic bacteria from gastric acidity, the infective dose of *Salmonella* could be 10 cells or less (D'Aoust and Maurer, 2007).

The micro-organisms causing different categories of food-borne illnesses associated with fish and fishery products are indicated in Table 11.

TABLE 11
Types of fish- and seafood-borne illnesses

Types of illness	Causative agent	
Infections	Bacterial infections	<i>Listeria monocytogenes</i> , <i>Salmonella</i> sp., <i>Escherichia coli</i> , <i>Vibrio vulnificus</i> , <i>Shigella</i> sp.
	Viral infections	Hepatitis A virus, norovirus, hepatitis E
	Parasitic infections	Nematodes (round worms), cestodes (tapeworms), trematodes (flukes)
	Toxi-infections	<i>Vibrio cholerae</i> , <i>Escherichia coli</i> , <i>Salmonella</i> sp.
Intoxications	Microbial	<i>Staphylococcus aureus</i> , <i>Clostridium botulinum</i>
	Biotoxins	Ciguatera, paralytic shellfish poisoning (PSP), diarrhoeic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), histamine
	Chemical	Heavy metals: mercury, cadmium, lead. Dioxins and polychlorinated biphenyls (PCBs). Additives: nitrites, sulphites

The natural habitat of the pathogenic bacteria varies and, based on their ecology, the bacteria may be grouped into three categories:

- bacteria indigenous to the aquatic environment (Table 12);
- bacteria indigenous to the general environment (Table 13);
- bacteria derived from animal/human reservoir (Table 14).

Bacteria that are indigenous to the aquatic environment and the general environment may be associated with fish at primary production stage (aquaculture or fish harvesting), and those derived from general environment or from the animal/human reservoir may be introduced as a result of contamination during handling and processing of fish. In either case, the initial levels of the bacteria are generally low and multiplication of the organism in fish to reach an infective dose or to produce toxin in fish precedes fish-borne illnesses. Therefore, for management of risk due to these pathogens, preventing their growth would be very important. Most of the pathogenic bacterial species have non-pathogenic environmental strains. For example, among *V. cholerae*, only those

belonging to serovar O1 and O139 cause the disease cholera (FAO/WHO, 2005b). Some strains of non-O1/non-O139 *V. cholerae* may cause gastroenteritis. Among *V. parahaemolyticus*, only a small percentage of environmental strains are pathogenic to humans. In some pathogens such as *V. vulnificus* and *L. monocytogenes*, it is currently not possible to distinguish pathogenic and non-pathogenic strains.

TABLE 12
Pathogenic bacteria indigenous to the aquatic environment

Organism	Distribution	Levels in fish at primary production stage
<i>Clostridium botulinum</i> Non-proteolytic types B, E, F	Worldwide, higher incidence in temperate waters	< 0.1–5.3 spores/g fish
<i>Vibrio cholerae</i>	Warm (> 15 °C) freshwater, estuarine and coastal environments	Generally low
<i>Vibrio parahaemolyticus</i> <i>Vibrio vulnificus</i>	Warm (> 15 °C) estuarine and coastal environments	Generally low, up to 10 ² –10 ³ /g
<i>Aeromonas</i> spp.	Warm (> 15 °C) freshwater, estuarine and coastal environments	Generally low
<i>Plesiomonas shigelloides</i>	Freshwater, worldwide	Generally low

Source: Modified from Huss (1997).

Pathogenic bacteria from the general environment may be found commonly in soil, dust, vegetation, water and on various food contact surfaces. These bacteria may be often present on fish, but mostly in small numbers.

TABLE 13
Pathogenic bacteria indigenous to the general environment

Organism	Distribution	Levels in fish at primary production stage
<i>Listeria monocytogenes</i>	Worldwide, soil, vegetation, silage, sewage, water	< 10 ² cfu/g in fish
<i>Clostridium botulinum</i> proteolytic type A, B	Worldwide, soil	Generally low
<i>Clostridium perfringens</i> Type A	Worldwide, soil	Generally low
<i>Bacillus cereus</i>	Worldwide, soil, vegetation, water	Generally low

Sources: Modified from Huss (1997) and Brenner *et al.* (2000).

Pathogenic bacteria derived from the animal/human reservoir (Table 14) may survive in the environment and even multiply there. Therefore, adoption of GHPs may reduce but not eliminate the chances of contamination of fish with these pathogens. As the levels of bacteria present as a result of such contamination from the environment are generally low, growth in fish before consumption is required to cause disease in humans. Thus, in addition to GHPs, it is important to take measures to prevent multiplication of pathogens in fish before consumption.

TABLE 14
Pathogenic bacteria in the animal/human reservoir

Organism	Distribution	Levels in fish at primary production stage
<i>Salmonella enterica</i> subspecies I	Worldwide, warm-blooded animals	Absent or generally low
<i>Salmonella enterica</i> subspecies II–V	Worldwide, cold-blooded animals and environment	Absent or generally low
<i>Shigella</i> spp.	Worldwide, humans and few primates	Absent or generally low
Pathogenic <i>Escherichia coli</i> (EPEC, ETEC, EAEC, EIEC)	Worldwide, warm-blooded animals	Absent or generally low
<i>Campylobacter</i> spp.	Worldwide, warm-blooded animals	Absent or generally low
<i>Staphylococcus aureus</i>	Worldwide, warm-blooded animals	Absent or generally low

Pathogenic bacteria are discussed in detail in the following sections.

3.2.1.1 *Vibrio* spp.

More than 80 species have been included in the genus *Vibrio*, of which at least 12 are capable of causing human infections (Oliver and Kaper, 2007). Members of this genus are Gram-negative curved or straight rods motile by polar flagellum. *Vibrio* spp. ferment glucose without producing gas, and most species produce oxidase and catalase. *Vibrio* spp. are commonly isolated from estuarine, coastal marine environments (some species such as *Vibrio cholerae* are found in freshwater) all over the world and seafood-borne illnesses are primarily caused by *Vibrio parahaemolyticus*, *V. vulnificus* and *V. cholerae* (FAO/WHO, 2003a). Of these, *V. parahaemolyticus* and *V. cholerae* cause gastrointestinal disease, while *V. vulnificus* causes septicemia. In the United States of America, the incidence of food-borne *Vibrio* infections increased in 2006 to the highest level since the FoodNet surveillance programme began (CDC, 2007), and the infections are most often associated with consumption of raw oysters. The emergence of a pandemic strain of *V. parahaemolyticus* (Nair *et al.*, 2007) and outbreaks of illness in Alaska, the United States of America, (McLaughlin *et al.*, 2005) and Chile (Cabello *et al.*, 2007) have led to increased interest among seafood safety managers in *Vibrio* spp. Table 15 indicates *Vibrio* spp. associated with human infections, both intestinal and extra-intestinal.

TABLE 15
Human pathogenic *Vibrio* spp.

Species	Occurrence in intestinal infections	Occurrence in non-intestinal infections
<i>V. cholerae</i> O1/O139	++++	+
Non-O1/O139 <i>V. cholerae</i>	++	++
<i>V. parahaemolyticus</i>	++++	+
<i>V. vulnificus</i>	+	+++
<i>V. fluvialis</i>	++	–
<i>V. furnissi</i>	++	–
<i>V. mimicus</i>	++	+
<i>V. hollisae</i> (now reclassified as <i>Grimontia hollisae</i>)	++	–
<i>V. metschnikovii</i>	+	+
<i>V. alginolyticus</i>	–	++
<i>V. carchariae</i>	–	+
<i>V. cincinnatiensis</i>	–	+
<i>V. damsela</i>	–	+

Note: + indicates frequency of occurrence in clinical samples
Source: Modified from FAO/WHO (2001).

3.2.1.2 *Vibrio parahaemolyticus*

Epidemiological aspects: Since its first recognition as a food-borne pathogen in Japan in the 1950s, *V. parahaemolyticus* has been implicated in several outbreaks and cases of

gastroenteritis in different parts of the world (Joseph *et al.*, 1982). Early studies in Japan showed that 96 percent of clinical strains produce a thermostable direct haemolysin (TDH), while only 1 percent of the environmental strains produce this haemolysin (Sakazaki, Iwanami and Tamura, 1968). Subsequently, TDH-negative strains from clinical cases were found to produce a TDH-related haemolysin, TRH (Honda, Ni and Miwatani, 1988). Currently, strains producing TDH and TRH are considered pathogenic to humans. Diverse serotypes may be associated with human infections, but, recently, strains belonging to the O3:K6 serotype and its variants have been found to be the causative agent of several outbreaks in different countries (Nair *et al.*, 2007). Although several publications refer to these strains as “pandemic” strains, Nair *et al.* (2007) pointed out that this is misleading in the epidemiological sense, because although outbreaks have been reported from different continents (except Oceania), they have not affected exceptionally high proportions of the population. Nevertheless, strains belonging to this group show clonality in molecular typing methods such as arbitrarily primed polymerase chain reaction (PCR), ribotyping or pulsed-field gel electrophoresis (PFGE) and are characterized by presence of only *tdh* gene (and not *trh* gene), some mismatches in nucleotides in the *toxRS* gene, and presence of an open reading frame ORF8 derived from a filamentous bacteriophage f237 (Nair *et al.*, 2007).

In Japan, *V. parahaemolyticus* is one of the most common causes of gastroenteritis and, annually, 500–800 outbreaks affecting 10 000 people are reported (FAO/WHO, 2011b). This organism is the leading cause of food-borne illness in Taiwan Province of China, causing 197 outbreaks in the period 1986–1995 (Pan *et al.*, 1997) and accounted for 69 percent of the food-borne cases between 1981 and 2003 (Su and Liu, 2007). *V. parahaemolyticus* accounted for 31.1 percent of 5 770 food-borne outbreaks that occurred in China from 1991 to 2001 (Liu *et al.*, 2004). In the United States of America, FoodNet data indicate that the yearly estimates of food-related illness attributed to *V. parahaemolyticus* for 1996, 1997 and 1998 were approximately 2 700, 9 800, and 5 600, respectively, and 62 percent of these were due to raw oyster consumption (FDA, 2005). It is also estimated that, due to under-reporting, the number of cases was underestimated by a factor of 1:20. In the period 1997–98, more than 700 cases (4 major outbreaks) occurred in the Gulf Coast, Pacific Northwest and Atlantic Northeast regions (Su and Liu, 2007). The outbreak that occurred in Alaska in 2004 extended by 1 000 km the northernmost documented source of oysters that caused illness (McLaughlin *et al.*, 2005). An outbreak involving 177 cases was reported in Washington and British Columbia in 2006 (CDC, 2006a). Seafood-borne diarrhoea was rare in Chile until 1998, when about 300 clinical cases due to *V. parahaemolyticus* O3:K6 were reported (Gonzalez-Escalona *et al.*, 2005). However, the number of cases came down to fewer than 10 per year until it rose again in 2004 causing large outbreaks in the environs of Puerto Montt in southern Chile with approximately 1 500, 3 600 and 900 cases in 2004, 2005 and 2006, respectively (Fuenzalida *et al.*, 2007).

Only two cases involving oyster consumption have been reported from Australia (FAO/WHO, 2011b). In a hospital-based surveillance in Kolkata, India, in the period 2004–05, *V. parahaemolyticus* accounted for 1.2 percent of enteropathogens detected (NICED, 2006). In some parts of Thailand, *V. parahaemolyticus* appears to be a common cause of gastroenteritis. In 1999, 317 cases were recorded in just two hospitals in Hat Yai City (Laohaprertthisan *et al.*, 2003). Until a decade ago, *V. parahaemolyticus* infections were considered rare in Europe, but this could be because such infections are not notifiable. An outbreak involving oyster consumption in 64 people occurred in 1999 in Galicia, Spain (Lozano-Leon *et al.*, 2003), and an outbreak that affected 44 people and linked to imported seafood was reported from France in 1997 (Robert-Pillot *et al.*, 2004). A further outbreak involving 80 people was reported from Spain in 2004 (Martinez-Urtaza *et al.*, 2005). Strains belonging to the pandemic clone have been involved in sporadic cases in Europe. Analysis of

13 clinical isolates of *V. parahaemolyticus* obtained in the period 1997–2004 in France showed that five isolates (one isolated in 1997, 1998, 1999 and two in 2003) belonged to the O3:K6 serotype and had molecular features of the pandemic clone (Quilici *et al.*, 2005). In Spain, there were two clinical isolates of *V. parahaemolyticus* O3:K6 and one of O3:KUT that showed molecular features of the pandemic clone during an oyster associated outbreak in 2004 (Martinez-Urtaza *et al.*, 2005). One case of *V. parahaemolyticus* O3:K6 gastroenteritis was reported from Italy in 2007 and one in 2008 (Ottaviani *et al.*, 2010). The pandemic clone of *V. parahaemolyticus* has also been isolated from the Russian Federation (Smolikova *et al.*, 2001) and also from Mozambique, Africa (Ansaruzzaman *et al.*, 2005).

V. parahaemolyticus gastroenteritis has been associated with a variety of fish and shellfish. While oysters have been the most common source in recent outbreaks in the United States of America and Europe, there have been reports of involvement of other types of seafood, including clams, shrimp, lobster, crayfish, scallops, crabs and finfish. In Japan, implicated foods include sashimi, pieces of raw fish fillet (responsible for 26 percent of outbreaks), followed by sushi, vinegary rice balls with pieces of raw fish fillet (23 percent), shellfish (16 percent) and cooked seafood (12 percent) (Anon., 2000a). In one study in Thailand, mackerels were found to be an important source (Atthasampunna, 1974). In countries such as India, where seafood is generally consumed after cooking, cross-contamination in the kitchen could be the cause of outbreaks (Nair *et al.*, 2007). Outbreaks of *V. parahaemolyticus* gastroenteritis aboard two Caribbean cruise ships reported in 1974 and 1975 (Lawrence *et al.*, 1979) were most probably caused by contamination of cooked seafood by seawater from the ships' seawater fire systems. In 1972, an estimated 50 percent of 1 200 persons who attended a shrimp feast in Louisiana in the United States of America became ill with *V. parahaemolyticus* gastroenteritis (Barker and Gangarosa, 1974), and samples of uncooked shrimp tested positive for the organism. Three outbreaks occurred in Maryland in the United States of America in 1971 (Dadisman *et al.*, 1972), with steamed crabs being implicated in two of the outbreaks after cross-contamination with live crabs. The third outbreak was associated with crabmeat that had become contaminated before and during canning.

Gastroenteritis due to *V. parahaemolyticus* infection is usually a self-limiting illness of moderate severity and short duration, and the symptoms include explosive watery diarrhoea, nausea, vomiting, abdominal cramps, and less frequently headache, fever and chills (FAO/WHO, 2011b). On rare occasions, septicemia, an illness characterized by fever or hypotension and the isolation of the micro-organism from the blood, can occur, particularly in immunocompromised individuals. In these cases, subsequent symptoms can include swollen, painful extremities with haemorrhagic bullae, and the duration of illness can range from 2 hours to 10 days (FAO/WHO, 2011b).

Dose–response estimations have been made for *V. parahaemolyticus* based on data from human volunteers (FDA, 2005; FAO/WHO, 2011b). These suggest that there is a low risk (< 0.001 percent) of gastroenteritis following consumption of 10^4 cells of pathogenic *V. parahaemolyticus* and a high risk (50 percent) when 10^8 cells are consumed. However, in the outbreak that occurred in Alaska, the levels of *V. parahaemolyticus* in oysters in the farm were in the range 0.3–430 MPN/g and the strains predominantly belonged to the O6:K18 serotype (McLaughlin *et al.*, 2005). It is not known whether some strains and those belonging to the O3:K6 serovar have a lower infective dose.

Ecology and association with fish and fishery products: *Vibrio parahaemolyticus* is found in the estuarine and coastal environments in the tropical to temperate zones (Joseph *et al.*, 1982). This organism is considered to be part of the autochthonous microflora in these environments, and there is no correlation between the presence of this organism and faecal contamination of the environments (Kaneko and Colwell, 1977; Joseph *et al.*, 1982). *V. parahaemolyticus* has been isolated from

seawater, sediment, marine animals, plankton, various fish and shellfish species (Joseph *et al.*, 1982). The organism has been isolated from a number of fish species and is associated primarily with the intestinal contents (Nair, Abraham and Natarajan, 1980). Thus, *V. parahaemolyticus* is naturally present in shellfish (shrimp and molluscan shellfish) growing and harvesting areas. Certain areas may have more favourable environmental conditions that support the establishment, survival and growth of the organism such as temperature, salinity, zooplankton, tidal flushing (including low-tide exposure of shellfish) and dissolved oxygen (Amako *et al.*, 1987; Garay, Arnau and Amaro, 1985; Kaneko and Colwell, 1977; Venkateswaran *et al.*, 1990). In temperate waters, *V. parahaemolyticus* is often detected in warmer months, and the organism has been reported to survive in the sediment during winter (Kaneko and Colwell, 1977). However, in tropical waters, *V. parahaemolyticus* can be detected throughout the year (Natarajan, Abraham and Nair, 1980; Deepanjali *et al.*, 2005). While salinity and temperature are considered important factors influencing the prevalence and levels of *V. parahaemolyticus* in temperate waters, (Kaneko and Colwell, 1977; DePaola *et al.*, 2003), salinity appears to be the major factor in tropical waters (Deepanjali *et al.*, 2005). *V. parahaemolyticus* can grow in sodium chloride (NaCl) concentrations ranging from 0.5 to 10 percent with optimum levels between 1 and 3 percent (Colwell *et al.*, 1984). Adsorption of *V. parahaemolyticus* on to plankton- or chitin-containing materials occurs with higher efficiency under conditions of estuarine salinity (Kaneko and Colwell, 1975). In freshwater systems, the presence of this organism has been reported to be transient and dependent on a biological host (Sarkar *et al.*, 1985).

In shrimp, the levels range from undetectable to 10^4 /g, high counts being rare (Cann, Taylor and Merican, 1981; Karunasagar, Venugopal and Karunasagar, 1984), and, in finfish, levels of ~ 88 /g have been reported (Chan *et al.*, 1989). As oysters have been implicated in several outbreaks, the abundance of *V. parahaemolyticus* in oysters has been extensively studied. In oysters in the United States of America, the levels detected range from undetectable to 10^4 /g. On the Atlantic and Gulf coasts, only 5 percent of samples had counts exceeding 10^3 /g (Cook, Bowers and DePaola, 2002). In Alabama oysters, the levels in the summer of 1999 were in the range of 10^2 – 10^3 /g, and the high levels (10^4 /g) reported in shell stock in the market are attributed to post-harvest growth (De Paola *et al.*, 2003). Similar levels have been reported from oysters in India (Deepanjali *et al.*, 2005). In Hiroshima Bay, Japan, the prevalence was 69 percent with levels ranging from 10^1 – 10^3 /100 g (Ogawa *et al.*, 1989). With the availability of specific DNA probes, it is possible to enumerate total and pathogenic *V. parahaemolyticus* (DePaola *et al.*, 2003; Deepanjali *et al.*, 2005). In the United States of America, the mean pathogenic *V. parahaemolyticus* as a percentage of total *V. parahaemolyticus* ranged from 0.3 to 3.2 in different regions (FDA, 2005). In India, only 10.2 percent of oyster samples were positive for pathogenic strains and, in these, the mean percentage pathogenic was 3.62 (Deepanjali *et al.*, 2005). Ten percent of various shellfish tested in Japan were positive for pathogenic *V. parahaemolyticus* with counts in the range of < 3 – 93 /10 g (Hara-Kudo *et al.*, 2003).

In Japan, the prevalence and levels of *V. parahaemolyticus* in the imported frozen seafood sampled at Osaka port and imported fresh seafood sampled at Kansai international airport in the period 1998–2000 has been reported by Chowdhury *et al.* (2001). Out of 335 frozen samples examined, 65 samples (19 percent) were positive while 234/949 fresh seafood samples (25 percent) were positive. Tuna had the highest prevalence in several different species of fresh seafood, and shrimp had the highest prevalence in frozen seafood, while Spanish mackerel had a lower prevalence. Of the 1 298 *V. parahaemolyticus* strains isolated, 2 strains (0.15 percent) contained the *tdh* gene and 17 strains (1.3 percent) contained the *trh* gene.

Growth and survival in seafoods: *V. parahaemolyticus* is a mildly halophilic (NaCl range 0.5–10 percent, optimum 3 percent) mesophilic (growth temperature

range 5–43 °C, optimum 37 °C) organism (ICMSF, 1996). This organism can grow in a pH range of 4.8–11, with the optimum being 7.8–8.6. At optimum temperature, the doubling time in shrimp was 9–10 min, and at 18.3 °C it was 144 min (Katoh, 1965). At 20 °C, the doubling time was 34 min in raw shrimp and 28 min in cooked shrimp (Liston, 1974). Growth rates in a range of seafoods and tryptic soy broth with 2.5 percent NaCl have been recorded and summarized (ICMSF, 1996). These data indicate that moderate populations of 10^2 – 10^3 organisms/g on seafood can increase to $> 10^5$ organisms/g in 2–3 h at ambient temperatures between 20 °C and 35 °C (ICMSF, 1996). *V. parahaemolyticus* can grow at a water activity of 0.940–0.996, with 0.981 being optimal (ICMSF, 1996). The ability of *V. parahaemolyticus* to grow in raw fish/shellfish depends on the species. In the oyster *Crassostrea virginica*, Cook and Ruple (1989) reported that levels of *V. parahaemolyticus* increased at temperatures above 10 °C, but in most cases did not detect an increase during storage at 10 °C. After one day of storage at either 22 °C or 30 °C the levels of *V. parahaemolyticus* were 2–3 orders of magnitude higher than those at harvest. Gooch *et al.* (2002) reported a 50-fold increase in *V. parahaemolyticus* levels after storage at 26 °C for 10 h and a 790-fold increase after 24 h. After refrigeration at 3 °C for approximately 14 days, a 6-fold decrease in the levels was observed. The results from these studies indicate that *V. parahaemolyticus* can grow rapidly in unrefrigerated oysters. However, Eyles, Davey and Arnold (1985) found that *Vibrio parahaemolyticus* grew poorly or not at all during storage of unopened Sydney rock oysters (*Crassostrea glomerata*) at 15 °C and 30 °C for 2 and 7 days. Although *V. parahaemolyticus* counts often increased at 30 °C, counts above 10^4 /g were not observed. A mathematical model to predict the growth rate of *V. parahaemolyticus* over a range of temperature and water activity conditions has been developed by Miles *et al.* (1997), which was used in the FAO/WHO risk assessment for *V. parahaemolyticus* in raw oysters (FAO/WHO, 2011b). Studies conducted in Japan show that in unshucked round clams and turban clams, *V. parahaemolyticus* did not grow at 10 °C and 25 °C, but in the meat of round clams, the counts increased by one log at 25 °C in 6 h (FAO/WHO, 2011b).

In the United States of America, the National Shellfish Sanitation Program stipulates that commercial shellfish must be refrigerated within 10 h after harvest, when the water temperature exceeds 27 °C (Drake, DePaola and Jaykus, 2007). The commercial cooling of oyster sacks has been estimated to take an average of 5.5 h (FDA, 2005) and some multiplication of *V. parahaemolyticus* might occur during this cooling time. Cook, Bowers and DePaola (2002) noted that *V. parahaemolyticus* levels in retail oysters were 1–2 log₁₀ greater than at harvest. A number of studies indicate that *V. parahaemolyticus* dies when exposed to temperatures < 5 – 7 °C, with the highest mortality rate being in the range 0–5 °C (ICMSF, 1996). Freezing combined with frozen storage for 30 days at -30 °C and -15 °C is projected to result in a 1.2- and 1.6-log₁₀ reduction of *V. parahaemolyticus* numbers in oysters, respectively. A similar decline (2–3-log₁₀) of *V. parahaemolyticus* (natural population and dosed with pathogenic O3:K6 serotype) was observed in oysters frozen for 35 days at -20 °C (FDA, 2005). Both pathogenic and non-pathogenic strains have been observed to respond similarly to freezing (FDA, 2005). The United States Interstate Shellfish Sanitation Conference (ISSC) has accepted freezing combined with frozen storage as an acceptable means of post-harvest treatment to control *V. parahaemolyticus* and *V. vulnificus*, which should be validated and HACCP compliant according to Code of Federal Regulation 21 CFR 123 (Drake, DePaola and Jaykus, 2007).

V. parahaemolyticus is sensitive to heat, and the ISSC has accepted heat as a post-harvest treatment to control this organism in shellfish (Drake, DePaola and Jaykus, 2007). The reported D-value in crab homogenate is < 1.0 min at 65 °C, and 2.5 min at 55 °C (ICMSF, 1996). In clam homogenates, the D-value is even lower (0.35–0.72 min at 49 °C), which could be because of the sensitivity of the organism to acidic pH

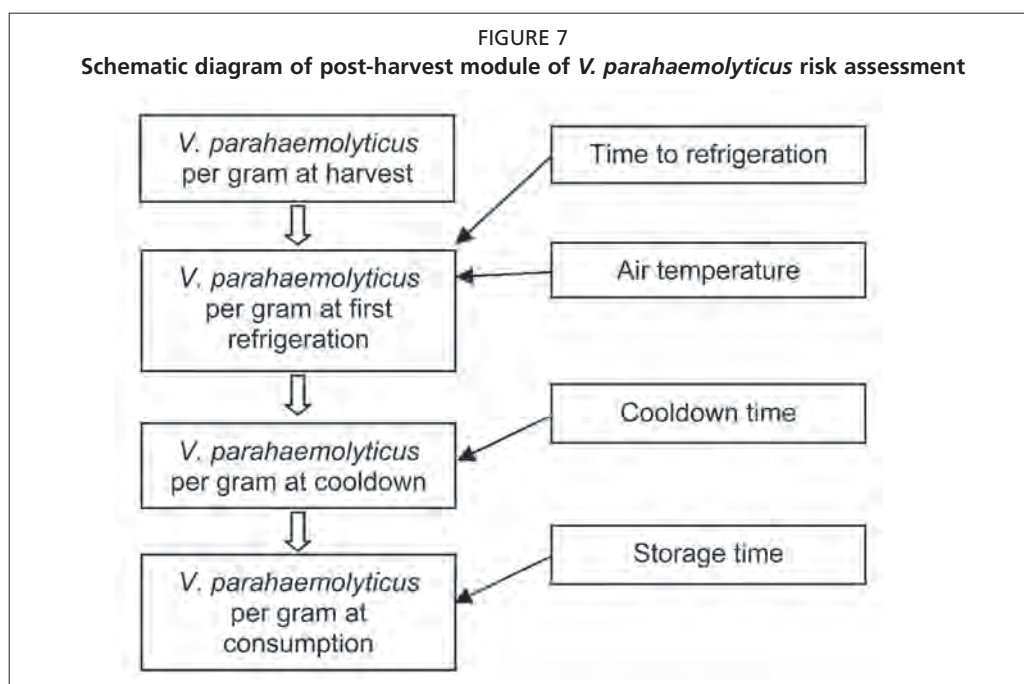
(ICMSF, 1996). Cook and Ruple (1992) observed a 6-log₁₀ reduction in *V. vulnificus* levels when shucked oysters were heated to an internal temperature of 50 °C for 5 min. *Vibrio parahaemolyticus* and *V. vulnificus* have been reported to have similar sensitivity to heat (FDA, 2005). Other studies have shown that a 4.5–6-log₁₀ reduction in *V. parahaemolyticus* densities could be expected by treating shucked oysters for 5 min at 50 °C (FDA, 2005). However, these studies observed that there is substantial variability in heat resistance among different strains. For example, when strains of serotype O3:K6 in phosphate buffered saline solution (PBS) were subjected to a mild heat treatment, there was a ~2-log₁₀ reduction. However, when non-O3:K6 pathogenic strains were treated similarly, a much greater reduction (~6-log₁₀) was observed (FDA, 2005).

Vibrios are sensitive to high hydrostatic pressure, and high-pressure treatment is emerging as a promising technology for control of pathogens in foods. D-values of 5.1 min and 4.0 min for *V. parahaemolyticus* cells treated with 170 MPa (10 atm = 1 megaPascal) at 23 °C in PBS and clam juice, respectively, have been reported (Styles, Hoover and Farkas, 1991). Various pathogenic vibrio species (approximately 10⁷ cfu/g) including *V. parahaemolyticus* were reduced to below detectable levels after 15 min at 250 MPa and 5 min at 300 MPa (Berlin *et al.*, 1999). After treatment for 30 s at 345 MPa, there was a 6-log₁₀ reduction in the level of *V. parahaemolyticus* resulting in < 10 cfu/ml. After 10 min at 240 MPa, the levels in the oysters ranged from < 10 cfu/ml to ~30 cfu/ml (Calik *et al.*, 2002). However, *Vibrio parahaemolyticus* strains vary in their resistance to high pressure with serotype O3:K6 strains being more resistant (Cook, 2003). For this serotype, the average reduction was approximately 6-log₁₀ after 5 min at 250 MPa in PBS with a range of from 5-log₁₀ to > 9.6-log₁₀, while for pathogenic strains of other serotypes the average log₁₀ reduction under the same conditions was ~12-log₁₀ reduction with a range of from 9.6-log₁₀ to > 15-log₁₀ (Cook, 2003).

Relaying is a process in which bivalve molluscs are removed from a microbiologically contaminated growing area to an acceptable growing or holding area under the supervision of the agency having jurisdiction and holding them there for the time necessary for the reduction of contamination to an acceptable level for human consumption (CAC, 2008a). This is not effective for *V. parahaemolyticus* as the organism is ubiquitous in the estuarine and coastal environments. Depuration is the process in which the reduction of micro-organisms to a level acceptable for direct consumption is achieved by the process of holding live bivalve molluscs for a period under approved, controlled conditions in natural or artificial seawater suitable for the process, which may be treated or untreated (CAC, 2008a). Depuration has been generally reported to have no significant effect on decreasing the level of *Vibrio* spp. in naturally infected oysters or clams, and some reports indicate that these microbes may even multiply in depurating shellfish, tank water, and plumbing systems (Eyles and Davey, 1984; Greenberg, Duboise and Palhof, 1982). However, reductions have been observed by some investigators, e.g. a 1-log₁₀ reduction in *V. parahaemolyticus* in the hard-shell clam, *Mercinaria mercinaria*, after 72 h of depuration at room temperature and > 2-log₁₀ reduction at 15 °C (Greenberg, Duboise and Palhof, 1982); a 5-log₁₀ reduction in laboratory-infected oysters (Son and Fleet, 1980).

Risk assessments: The Food and Drug Administration (FDA, 2005) and FAO/WHO (2011b) have carried out quantitative risk assessment of *Vibrio parahaemolyticus* in raw oysters. In the FDA risk assessment, based on data available in the United States of America, a model for predicting *V. parahaemolyticus* levels in oysters based on water temperature was developed. The post-harvest oyster handling practices in the United States of America and the effect of these practices on levels of *V. parahaemolyticus* were modelled. Growth of *V. parahaemolyticus* in American oysters at 26 °C reported by Gooch *et al.* (2002) and the model developed by Miles *et al.* (1997) for estimating

growth rate at different temperatures in broth were used to model the growth in oysters in the post-harvest module. Data from two regions in the United States of America (Pacific Northwest and Gulf coast) were used to estimate the proportion of strains that are pathogenic. It was estimated that about 50 percent of oysters are consumed raw and that each serving would be about 200 g. The FDA risk assessment predicted the mean annual number of illnesses to be the highest in the Gulf coast region with 1 406, 132, 7 and 505 cases occurring in summer, autumn, winter and spring, respectively. The current ISSC/FDA guideline recommends that if *V. parahaemolyticus* levels in oysters exceed 5 000/g, they have to be tested for pathogenic (tdh+) strains, and if positive, harvesting is to be closed (FDA, 2005). The risk assessment estimated risk reductions that can be achieved by having a control plan based on levels of *V. parahaemolyticus* at harvest in oysters. A standard of 5 000/g *V. parahaemolyticus* at the time of harvest could (potentially) eliminate 28 percent of the illnesses associated with the consumption of oysters from the Gulf coast region, with 6 percent of the harvest having to be diverted from the “raw market” (FDA, 2005). The risk assessment suggests that in the absence of subsequent post-harvest mitigations, “at harvest” guidance levels of 10^5 , 10^3 , 10^2 total *V. parahaemolyticus* per gram could potentially reduce the illness rate by 1.6, 68 and 98 percent with corresponding impacts of 0.25, 21 and 66 percent of the harvest, respectively. If the control is applied on the basis of *V. parahaemolyticus* levels at retail, a standard to 10^4 /g would reduce illness by 99 percent and 43 percent of the harvest would have to be diverted from the raw market. A 5 000/g standard could almost eliminate almost 100 percent of illnesses, with 70 percent of the harvest having to be diverted from the raw market (FDA, 2005).



Source: FAO/WHO (2011b).

The FAO/WHO risk assessment of *V. parahaemolyticus* in raw oysters used a similar approach (Figure 7) to estimate risk of illness in Australia, New Zealand, Canada and Japan (FAO/WHO, 2011b). Local data on water and air temperature, local harvest practices and prevalence of *V. parahaemolyticus* in oysters in these countries were used: also used were data from the United States of America on proportion of pathogenic *V. parahaemolyticus*, multiplication of *V. parahaemolyticus* in oysters,

consumption patterns and under-reporting of illness. The model predicts an annual incidence of 91 cases for Australia, 66 for Japan and 186 for Canada. Epidemiological data indicate that there were only 2 cases in Australia in 18 years, and 212 cases in 10 years (1997–2006) in Canada (FAO/WHO, 2011b). The overestimation of illness could be due to several factors such as growth of *V. parahaemolyticus* in different oyster species (no growth reported for Sydney rock oysters even at ambient temperatures), presence and proportion of pathogenic *V. parahaemolyticus* and under-reporting factors in the model used for the United States of America. The risk assessment also estimated the impact of three different limits for *V. parahaemolyticus*: 100 cfu/g, 1 000 cfu/g and 10 000 cfu/g applied when the products are cooled after harvesting, when the population of *V. parahaemolyticus* has stabilized, i.e. when the temperature becomes too low for further growth but not so low that die-off occurs (Table 16). At the standard of 100/g, a 99 percent reduction in illness in Australia and Japan can be achieved with a diversion of 67 percent and 16 percent of oysters from raw markets, respectively (FAO/WHO, 2011b). This shows that the impact of implementation of criteria could be diverse in different geographical regions.

TABLE 16
Reduction in illness, based on meeting specified target numbers of *V. parahaemolyticus*, together with commensurate rejection of product for raw consumption

Specified target	Reduction (%) in the number of predicted illnesses			Product (%) rejected to achieve these reductions in illnesses		
	Australia (summer)	New Zealand (summer)	Japan (autumn)	Australia (summer)	New Zealand (summer)	Japan (autumn)
100 cfu/g	99	96	99	67	53	16
1 000 cfu/g	87	66	97	21	10	5
10 000 cfu/g	52	20	90	2	1	1

Source: FAO/WHO (2011b).

FAO and the WHO have also carried out risk assessment of *V. parahaemolyticus* in raw and undercooked finfish (FAO/WHO, 2011b). The assessment was done for Japanese horse mackerel (*Trachurus japonicus*) because 282 000 tonnes of horse mackerel is harvested in Japan annually and 30 percent of this is consumed raw as sushi or sashimi in Japan. Japanese published and unpublished data on the prevalence and concentration of *V. parahaemolyticus* in horse mackerel at different steps in the food chain were used in the risk assessment. The average consumption weight per serving was estimated to be 73 g. It was assumed that the levels of *V. parahaemolyticus* on the surface of the fish and in the intestines at harvest were proportional to that of the gill, with the ratio calculated from the data reported in one of the Japanese studies (FAO/WHO, 2011b). The *V. parahaemolyticus* numbers were estimated for different scenarios: no washing, washing fish in clean water, washing fish in water that contains *V. parahaemolyticus*. The probability of becoming ill per serving of raw horse mackerel was estimated to be 8.77×10^{-7} (best scenario) to 3.75×10^{-5} (worst scenario). The estimated number of cases per year would be 70–3 000 in Japan.

Yamamoto *et al.* (2008) reported quantitative risk assessment of *V. parahaemolyticus* in bloody clams (*Anadara granosa*) in southern Thailand. The prevalence and concentration of *V. parahaemolyticus*, and the proportion of pathogenic strains in bloody clam at harvest and retail were estimated by the MPN-PCR method. This study estimated the illness rate to be 6–10 000 persons/year.

Risk management strategies: Currently available risk assessments indicate that there are wide geographical variations in the predicted number of illnesses from *V. parahaemolyticus* owing to a number of factors such as prevalence and levels of total and pathogenic strains, post-harvest and consumption practices. Even for a single commodity, e.g. raw oysters, the predicted level illness even within a country

(e.g. the United States of America) varies in different regions. Therefore, it would not be possible to suggest a globally applicable microbiological criterion even for raw oysters. However, considering the outputs of various risk assessments, the Recommended Code of Hygienic Practice for *Vibrio* spp. in Seafood has an annex on control measures for *V. parahaemolyticus* and *V. vulnificus* in raw bivalve molluscs (CAC, 2010).

3.2.1.3 *Vibrio vulnificus*

Epidemiological aspects: *Vibrio vulnificus* is a common inhabitant of warm-water estuarine environments all over the world. Currently, three biotypes are recognized based on a combination of phenotypic, serologic and host-range characters (Drake, DePaola and Jaykus, 2007). Biotype 1 strains are indole positive, serologically diverse and are associated with human infections. Biotype 2 strains are indole negative and considered mainly as eel pathogens, but may also be opportunistic human pathogens, being associated with infections in eel handlers. This biotype has three serotypes, and strains associated with eel and human infections belong to serotype E (Sanjuan and Amaro, 2004). Biotype 3 has five atypical biochemical characters, genetically clonal and has been isolated from 62 Israeli patients with wound infection or septicemia. This biotype has not been associated with food-borne infections (Drake, DePaola and Jaykus, 2007). The virulence of this organism seems to be related to multiple factors such as presence of a polysaccharide capsule, ability to obtain iron from transferrin, ability to produce extracellular enzymes and exotoxin (Drake, DePaola and Jaykus, 2007). Most of the virulence-associated factors are present in more than 95 percent of environmental strains. Recent molecular studies suggest that it may be possible to differentiate clinical and environmental strains. Rosche, Yano and Oliver (2005) using nucleotide sequence analysis showed that Biotype 1 strains could be distinguished into two types that strongly correlate with clinical (C) or environmental (E) origin. C-genotypes showed greater resistance to human serum than E-genotypes and had lower LD₅₀ suggesting that C-genotype strains may be more virulent (Bogard and Oliver, 2007; Rosche, Binder and Oliver, 2010). While similar levels of C- and E-genotypes were found in estuarine waters, oysters had 85 percent E-genotypes (Warner and Oliver, 2008).

The disease rarely (< 5 percent) occurs in healthy individuals, and liver disease (including cirrhosis due to alcohol consumption) is a risk factor for *V. vulnificus* infection. Other predisposing factors are diabetes, gastrointestinal disorders (ulcer, surgery), haematological conditions, and immunocompromised condition associated with cancer and therapy with immunosuppressive drugs. The fatality rate (about 50 percent) is the highest among food-borne pathogens (FAO/WHO, 2005c). However, the attack rate is low, with one illness occurring per 10 000 meals of raw United States Gulf coast oysters (containing *V. vulnificus*) served to the highest-risk population, i.e. people with liver diseases (FAO/WHO, 2005c). The incubation period ranges from 7 h to 10 days, with symptoms appearing within 36 h in most cases (Oliver and Kaper, 2007). Most patients present with sudden onset of fever and chills, generally accompanied with nausea, vomiting, abdominal pain, hypotension (systolic pressure <85 mm). In more than 60 percent cases, secondary lesions appear, mostly on the legs that often develop necrotizing fascitis or vasculitis that may require surgical debridement or amputation (Strom and Paranjapaye, 2000; Oliver and Kaper, 2007). *V. vulnificus* can be isolated from blood and cutaneous lesions. Epidemiological data suggest that men are more susceptible than women to *V. vulnificus* infection (Shapiro *et al.*, 1998; Merkel *et al.*, 2001). Rare cases of atypical infections have been reported, and these include septic arthritis (Johnson and Arnett, 2001), meningoencephalitis (Kim *et al.*, 2003a) and ocular infection (Jung *et al.*, 2005) following consumption of raw oysters or raw fish. Apart from primary septicemia, *V. vulnificus* may be associated with wound infections, and Strom and Paranjapaye (2000) noted that 69 percent of such

infections were associated with occupational exposures among oyster shuckers and commercial fishers. Wound infections may progress to echymoses, cellulitis, bullae and necrotizing fasciitis, but the mortality rate (25 percent) is much lower than in the case of primary septicaemia (Jones and Oliver, 2009), but 50 percent of cases may require surgical debridement or amputation. *V. vulnificus* infection may also result in mild gastroenteritis with vomiting, diarrhoea and abdominal cramps.

It is estimated that about 100 cases of primary septicaemia due to *V. vulnificus* occur per year in the United States of America (Drake, DePaola and Jaykus, 2007). The Korean Center for Disease Control estimates 40–70 confirmed cases per year, and this high rate is suspected to be due to consumption of raw seafood or higher prevalence of predisposing factors (Drake, DePaola and Jaykus, 2007). However, in Japan, Inoue *et al.* (2008) estimated 12–24 cases per year, and in Taiwan Province of China, there was a peak occurrence in 2000 with 26 cases per million of the population (Hsueh *et al.*, 2004). In Japan, oysters are not the primary source, as raw oysters are eaten only in winter and most infections occur in the period June–November with a peak in July. A mud shrimp, *Upogebia major*, was the common agent associated with *V. vulnificus* infections (Inoue *et al.*, 2008). Most cases occurred in western Japan, with about 50 percent of cases occurring in Kyushu. In Japan, 72.3 percent of infections had septicaemia, and the mortality rate was 75 percent. Most patients (86.5 percent) had liver function impairment, with 56.9 percent having liver cirrhosis and 10.1 percent liver cancer (Inoue *et al.*, 2008). Three cases in Kumamoto Prefecture, Japan, were attributed to consumption of raw flathead fish that had been marinated in soy sauce for one day (Ono, Inoue and Yokoyama, 2001). In Europe, *V. vulnificus* infections are rare and mostly wound infections (Baker-Austin *et al.*, 2010). Rare cases of septicaemia have been reported from Thailand (Thamlikitkul, 1990) and India (Saraswathi, Barve and Deodhar, 1989).

Ecology and association with fish and fishery products: *Vibrio vulnificus* is a natural inhabitant of warm estuarine and coastal environments throughout the world. The organism has been isolated from waters where the temperatures range from 9–31 °C and proliferates in waters where temperature exceeds 18 °C (Kaspar and Tamplin, 1993; Strom and Paranjpaye, 2000). Although this organism has worldwide distribution and has been isolated from coastal marine and estuarine waters, sediment, plankton, various shellfish (both molluscan and crustacean) and finfish species (FAO/WHO, 2005c; Drake, DePaola and Jaykus, 2007), detailed ecological studies have been done only from a few countries. The abundance varies considerably and is greatly influenced by temperature and salinity. In North America, higher densities are observed in mid-Atlantic, Chesapeake Bay and Gulf coast waters, where temperatures are warmer throughout the year, while densities are lower in Pacific, Canadian and North Atlantic waters (Kaysner *et al.*, 1987; O’Neil, Jones and Grimes, 1992; DePaola, Capers and Alexander, 1994; Wright *et al.*, 1996; Motes *et al.*, 1998). The lowest temperature at which *V. vulnificus* has been isolated varies geographically, being 8 °C at Chesapeake Bay (Wright *et al.*, 1996) and < 12.5 °C in Gulf coast waters (Simonson and Siebeling, 1986). The organism survives in sediment during winter. In tropical waters, where temperature does not go below 18 °C, abundance of *V. vulnificus* is influenced by salinity (Parvathi *et al.*, 2004). In south India, the highest *V. vulnificus* levels were found during the monsoon season when the salinities were less than 5 ppt, and these organisms were not detectable at salinities exceeding 25 ppt (Parvathi *et al.*, 2004). Salinity has a significant effect on the abundance of the organism even in temperate waters. In the waters of the United States of America, numbers of *V. vulnificus* were high at salinity levels of 5–25 ppt, but dropped by 58–88 percent at salinities more than 30 ppt (Kaspar and Tamplin, 1993; Motes *et al.*, 1998). *V. vulnificus* can colonize plankton and fish gut (DePaola, Capers and Alexander, 1994; Wright *et al.*, 1996; FAO/WHO, 2005c). *V. vulnificus* produces chitinase, which might help the organism

to colonize zooplankton (Strom and Paranjapaye, 2000). Through fish, the organism even reaches the gut of birds, as Miyasaka *et al.* (2005) found 14.1 percent aquatic birds in Japan to be positive for *V. vulnificus*.

Levels of *V. vulnificus* in oysters could be 100 times higher than in the waters surrounding them. On the United States Gulf coast, the levels in oysters may reach 10^4 cfu/g during the summer months (Drake, DePaola and Jaykus, 2007), and in tropical waters of India, similar levels were reached in oysters when salinities were less than 10 ppt (Parvathy *et al.*, 2004). *V. vulnificus* counts exceeding 10^6 /g have been reported from the intestines of benthic fish inhabiting oyster reefs (DePaola, Capers and Alexander, 1994). There is no correlation between the prevalence or occurrence of *V. vulnificus* and faecal contamination of waters (Tamplin *et al.*, 1982; Parvathi *et al.*, 2004), hence faecal coliforms and/or *Escherichia coli* cannot be used as indicator organisms for this pathogen.

Growth and survival in seafoods: *V. vulnificus* does not grow in oysters at temperatures below 13 °C and prolonged refrigeration could lead to reduction in numbers (Cook, 1994; Cook and Rupple, 1992). While Cook and Rupple (1992) noted that levels in refrigerated shellfish became non-detectable (< 3 /g) in 14–21 days, Kaysner *et al.* (1989) observed survival in artificially contaminated oysters for 14 days at 2 °C, suggesting that refrigeration cannot be relied upon for elimination of this pathogen in oysters. The rate of decline in refrigerated oyster shell stock has been estimated to be 0.041 log unit per day (Cook, Bowers and DePaola, 2002). In fact, if the temperature is not controlled immediately after harvest, growth of *V. vulnificus* in oyster could occur. Cook (1997) demonstrated that *V. vulnificus* levels in oyster shell stock held without refrigeration for 3.5, 7, 10.5 and 14 h increased 0.75, 1.3, 1.74 and 1.94 log units. It has also been reported that *V. vulnificus* levels in retail oysters originating from Gulf of Mexico were 1–2 log units greater than at harvest (Cook, Bowers and DePaola, 2002). It has been estimated that commercial cooling of oyster stocks could take an average of 5.5 h (FDA, 2005) and, therefore, the time shell stock is unrefrigerated on boat deck is an issue in control plans.

Four to five \log_{10} reductions in numbers of natural *V. vulnificus* population in oysters occur when frozen to –40 °C and stored for 3 weeks (Cook and Rupple, 1992). However, cold adaptation at 15 °C may reduce the effectiveness of freezing (Bryan *et al.*, 1999). A combination of vacuum packaging and freezing can bring down *V. vulnificus* counts by 3–4 \log_{10} units in 7 days but although numbers continue to decline until day 7, complete elimination cannot be achieved (Parker *et al.*, 1994).

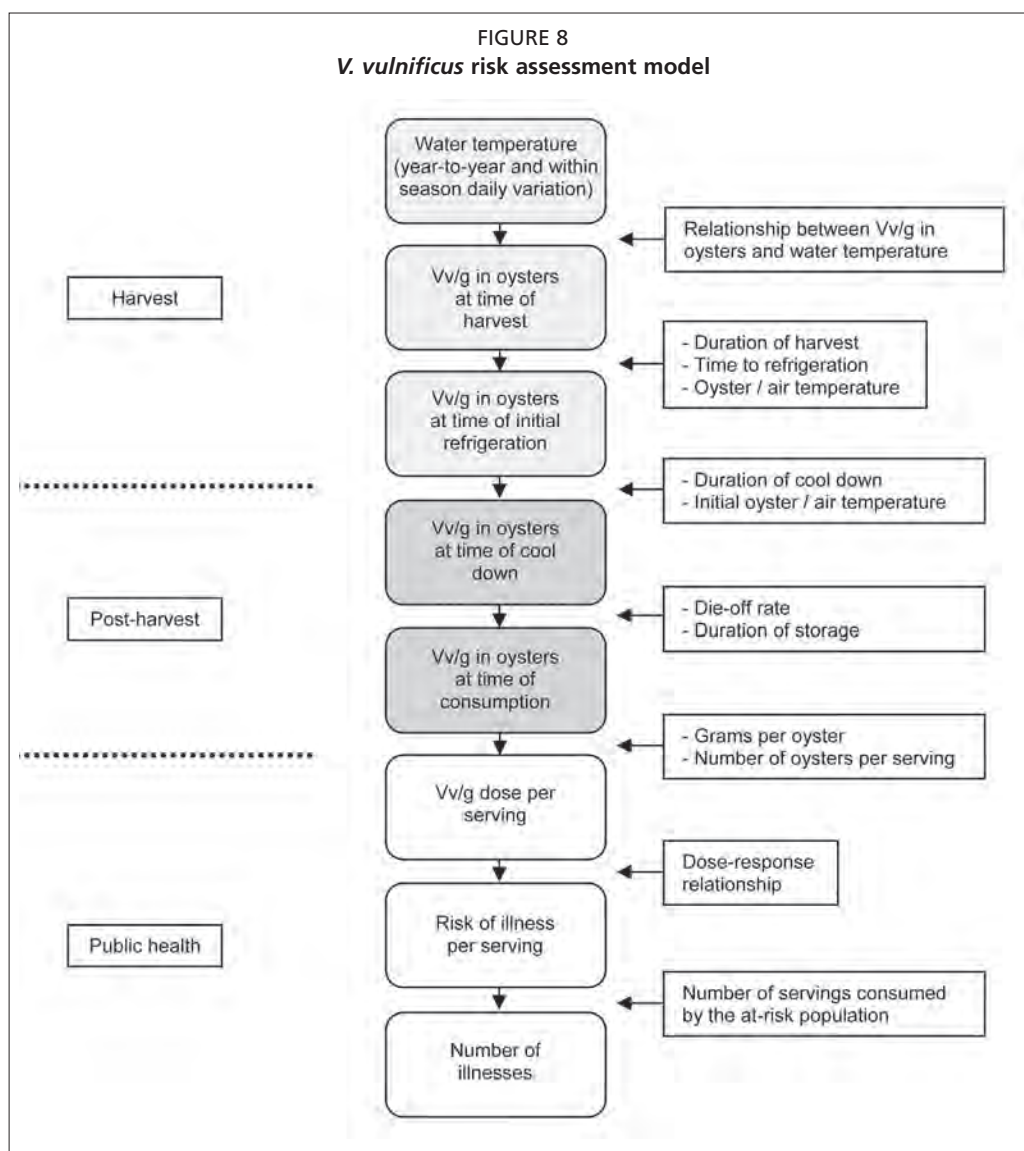
V. vulnificus is sensitive to heat with a 6 \log_{10} reduction in numbers occurring when subjected to 50 °C for 5 min in shucked oyster meat (Cook and Rupple, 1992). Natural populations of *V. vulnificus* (4.3×10^3 cfu/g) could be reduced to non-detectable levels by exposing them to 50 °C for 10 min (Cook and Rupple, 1992). D-values at 47 °C were 3.44–3.66 min for opaque colonies and 3.18–3.38 min for translucent colonies (Kim *et al.*, 1997). In North and South Carolina, the United States of America, commercial shell stock is subjected to heat shock by submerging batches of about 70 chilled oysters in wire baskets into a heat-shock tank containing about 850 litres of potable water at a temperature of 67 °C for about 5 min depending on oyster size and condition (Drake, DePaola and Jaykus, 2007). This process has been shown to reduce *V. vulnificus* levels by 2–4 \log_{10} units (Hesselman, Motes and Lewis, 1999). *V. vulnificus* cells were inactivated at pH 2.0 (Koo, DePaola and Marshall, 2000). *V. vulnificus* is sensitive to ionizing radiation, and irradiation doses of 1.0 kGy applied on whole shell oysters can reduce the cell numbers from 10^7 cfu/g to undetectable levels (Andrews, Jahncke and Millikarjunan, 2003). Hydrostatic pressure of 250 MPa for 120 s reduced *V. vulnificus* to $> 5 \log_{10}$ units in oyster (Cook, 2003).

Risk assessments: A quantitative risk assessment for *V. vulnificus* in raw oysters was documented by FAO/WHO (2005c), and this study modified the FDA

V. parahaemolyticus risk assessment model to assess the risk of *V. vulnificus* primary septicemia in the United States of America. The geographical coverage was limited because quantitative data for *V. vulnificus* levels in oysters at the point of consumption and the data for the susceptible population were available for only for the United States of America (FAO/WHO, 2005c). Data on *V. vulnificus* levels in oysters were based on weekly analysis of oysters from four Gulf states conducted in the period 1994–95 (Motes *et al.*, 1998, FAO/WHO, 2005c) and all strains were considered equally virulent. Although association of certain genotypes with clinical cases has been reported (Nilsson *et al.*, 2003), data on seasonal and regional distribution of such strains or on the ability of such strains to grow and/or survive in oysters under typical industry practices were not available. The model used for determining exposure assessment is illustrated in Figure 8. The harvest and post-harvest module were based on post-harvest practices (duration of oysters in harvest vessel in water, time to first refrigeration, cooldown time) derived based on surveys conducted on the Gulf coast. *V. vulnificus* growth in oysters, survival during refrigeration and levels at consumption were estimated based on data from studies along the Gulf coast of the United States of America (Cook, 1997; Cook, Bowers and DePaola, 2002). The model predicted that the mean *V. vulnificus* levels in oysters would be 5.7×10^4 /g in summer and 8.0×10^1 /g in winter. At a serving size of 196 g, the ingested dose would be 1.1×10^7 *V. vulnificus* in summer and 1.6×10^4 in winter. FDA data on the prevalence of risk factors in the United States of America population and oyster consumption data from surveys were used in the model (FAO/WHO, 2005c). The dose–response relationship was modelled by estimating the exposure per eating occasion and the number of eating occasions for oyster-associated *V. vulnificus* cases reported to the United States CDCs in the period 1995–2001. The model predicted 0.5, 11.5, 12.2 and 8 illnesses for winter (January–March), spring (April–June), summer (July–September) and autumn (October–December), respectively. When compared with epidemiological data, the numbers of reported cases (averages for 1995–2001 were 0.6 in winter, 9.6 in spring, 13.5 in summer and 7.4 in autumn) were within the 90 percent confidence limit predicted by the model (FAO/WHO, 2005c).

The risk assessment also predicted the reductions in illness that could be achieved by post-harvest treatments to reduce *V. vulnificus* levels to target values such as 3/g, 30/g or 300/g. In the United States of America, there are three validated methods to achieve end-point criterion of < 3 MPN/g *V. vulnificus* and these include mild heat treatment (50 °C), freezing with extended frozen storage, and high hydrostatic pressure. If all oysters were treated to achieve a target level of 3/g, the model predicted that the number of cases could be reduced from the current 32 reported cases per year to one case every 6 years. If the target were shifted to 30/g or 300/g, then the predicted cases would increase to 1.2 and 7.7 cases per year, respectively (FAO/WHO, 2005c). At a time to refrigeration range of 0–20 h, the predicted illness ranged from 17.7 to 59.3 cases, suggesting that immediate cooling of oysters alone is not adequate to achieve a substantial reduction in the number of *V. vulnificus* illnesses. As *V. vulnificus* levels in oysters harvested from waters with a salinity of > 30 ppt is greatly reduced, it is predicted that if all oysters were harvested from waters at a salinity of > 30 ppt, irrespective of the water temperature, *V. vulnificus* illnesses would be < 1 case per year (FAO/WHO, 2005c). Relaying oysters to high-salinity waters (> 32 ppt) has been shown to reduce *V. vulnificus* levels by 3–4 log units (< 10 /g) within 2 weeks. Based on the FAO/WHO risk assessment, the Codex Committee on Food Hygiene developed a code of hygienic practice for control of *Vibrio spp.* in seafood with an annex on control measures for *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs. This code recommends assessment of the need for control measures based on: (i) number of sporadic illnesses associated with bivalve molluscs in the area; (ii) water temperature at harvest, air temperature and harvest and post-harvest practices; and (iii) water

salinity at harvest. As there is wide geographical variation in the prevalence and levels of *V. vulnificus* in bivalves, control measures that have been validated and appropriate for the region may be adopted by the competent authority having jurisdiction and implemented under the HACCP system. Validation of control measures should be carried out in accordance with the Codex guidelines for the validation of food safety control measures (CAC/GL 69–2008).



Source: FAO/WHO (2005c).

Risk management: *V. vulnificus* resides inside various tissues of oysters; hence, depuration is ineffective in elimination of this pathogen. However, relaying oysters in high-salinity (> 30 ppt) waters for 17–49 days caused a decrease in population from 10^3 cfu/g to < 10 MPN/g (Motes and De Paola, 1996). The United States National Shellfish Sanitation Program (NSSP) guide (2009) includes the following strategies for minimizing the risk due to *V. vulnificus* in molluscan shellfish in states reporting two or more cases of *V. vulnificus* illness per year: (i) increased educational efforts targeted towards the population at risk to improve their awareness of the risks of eating raw molluscan shellfish and to change their eating behaviour to reduce or stop eating raw or untreated molluscan shellfish; (ii) limited harvest restrictions on areas incriminated

in outbreaks; (iii) requirement for the temperature of shell stock to be brought down to 10 °C or less by using ice, mechanical refrigeration or other means within specified period (10 h when water temperature is > 28 °C; 12 h when water temperature is > 23 °C up to 28 °C; 14 h when water temperature is 18–23 °C and 36 h when water temperature is < 18 °C); and (iv) phased-in post-harvest treatment requirements or other controls.

3.2.1.4 *Vibrio cholerae*

Epidemiological aspects: *Vibrio cholerae* is a heterogeneous species consisting of more than 220 serotypes. The disease cholera is caused only by serotypes O1 and O139. These are also referred to as cholerae *V. cholerae*. Strains belonging to non-O1/non-O139 serotypes of *V. cholerae* are widely distributed in the aquatic environment and they are mostly not pathogenic to humans, although they may occasionally be associated with sporadic cases of gastroenteritis (Kaper, Morris and Levine, 1995; Desmarchelier, 1997). The O1 serovar is classified into three antigenic forms: Inaba, Ogawa and Hikojima. *V. cholerae* O1 strains are classified into two biotypes, Classical and El Tor, based on their phenotypic characteristics (Kaper, Morris and Levine, 1995). Recent studies have shown that the Classical biotype strains are rarely isolated from any part of the world (Sack *et al.*, 2003). The severe form of the disease, termed cholera gravis, is characterized by passage of voluminous stools of rice water character leading to dehydration, hypovolemic shock, acidosis, and, unless appropriate treatment is initiated, death. However, it has been estimated that only 2 percent of those infected with El Tor biotype and 11 percent of those infected with Classical biotype develop severe disease. Five percent of El Tor infections and 15 percent of Classical infections may result in moderate illness that can be managed in outpatient clinics (Kaper, Morris and Levine, 1995). Symptoms due to O1 and O139 serotypes appear to be identical. The most important virulence factor associated with *V. cholerae* O1 and O139 is the cholera toxin. The *ctx* genes (*ctxA* and *ctxB*) encoding the production of the cholera toxin have been sequenced, and this has enabled development of DNA probes and PCR methods for detection of this gene in the isolates of *V. cholerae* O1 and O139 (Shirai *et al.*, 1991; Koch *et al.*, 1993; Karunasagar *et al.*, 1995). The cholerae El Tor biotype strains of *V. cholerae* are grouped in four major clonal groups: (i) the seventh pandemic, (ii) the United States Gulf Coast, (iii) Australia; and (iv) Latin America. These seem to reflect broad demographic and epidemiological associations (Wachsmuth *et al.*, 1994).

Cases of cholera occur in several countries in Asia, Africa and also occasionally in the United States of America, where the organism is present in the Gulf coast (Oliver and Kaper, 2007). Ingestion of contaminated water or food has been the cause of most outbreaks, and fish and fishery products are occasionally incriminated. A variety of fish and fishery products have been involved in outbreaks of cholera in different parts of the world (FAO/WHO, 2005a). Crustaceans, molluscs and finfish prepared in a variety of forms have been vectors for the transmission of *V. cholerae*. Transmission of *V. cholerae* by seafood can be acute where fish and shellfish are consumed raw (DePaola, 1981). Seventy-five of 336 passengers on an airliner were affected in the Americas in 1992 in a case in which cold seafood salad was implicated (Eberhart-Phillip *et al.*, 1996). The shellfish most often associated with cholera cases are molluscan shellfish (oysters) and crabs. While oysters are consumed raw in many countries, crabs are generally cooked, although even after boiling crabs for up to 10 min or steaming for up to 30 min, *V. cholerae* O1 may still retain viability (Blake *et al.*, 1980). There are also a few outbreaks linked to crustacean shellfish: one outbreak linked to the consumption of raw shrimp in the United States of America in 1986, where the source was domestic; an outbreak in Japan in 1978 associated with lobsters imported from Indonesia; and an outbreak linked to the consumption of raw shrimp in the Philippines

in 1962. However, in most cases, it is not possible to assess whether *V. cholerae* O1 was naturally present or cross-contaminated after harvest (FAO/WHO, 2005b). Finfish have also occasionally been involved, e.g. reef fish in Guam (Haddock, Truong and Aguon, 2002); unspecified fish brought into Germany by a Nigerian (Schurmann *et al.*, 2002); and whitebait from Indonesia in cases in Sydney (Forssman *et al.*, 2007).

Severe diarrhoea due to *V. cholerae* O75 has been reported in the United States of America, although this has not caused large outbreaks. Between 2003 and 2007, *V. cholerae* O75-producing cholera toxin was isolated from six patients with severe diarrhoea and, in some cases, raw oysters were linked to the infections (Tobin-D'Angelo *et al.*, 2008). A further ten cases linked to raw or lightly cooked oyster consumption were reported in Florida in 2011, but none of the cases required rehydration therapy (Onifade *et al.*, 2011). Although *V. cholerae* O75 isolated from these cases produced cholera toxin, the disease was milder than cholera. Although *ctx*-positive non-O1 and non-O139 strains have been found, these strains often lack the full set of virulence genes found in epidemic strains. Chakraborty *et al.* (2000) noted absence of *tcpA* genes in *ctx*-positive strains, while Rivera *et al.* (2001) noted absence of genes encoding zonula occludens toxin (*zot*). A multiplex PCR amplifying *tcp* and *ctx* gene has been suggested for detection of cholerae *V. cholerae* O1/O139 from aquatic ecosystems for cholera surveillance programmes (Rivera *et al.*, 2003).

Ecology and association with fish and fishery products: The primary source of *V. cholerae* O1 and O139 is the faeces of persons acutely infected with the organism. The organism reaches water most often through sewage. The presence of the organism in the aquatic environment is not directly correlated with the presence of faecal coliform bacteria, but nutrients discharged with human sewage may enhance the survival of *V. cholerae*. The organism can survive in waters for long periods, and there are several instances where water has been implicated by epidemiological studies as a vehicle of *V. cholerae* O1. The survival time of *V. cholerae* in water has been estimated and the average time for a 1-log decline in cell number (t_{90}) is a function of the organism as well as the biotype (Feachem, Miller and Drasar, 1981). The work of Colwell and co-workers has shown that *V. cholerae* O1 can survive in water almost indefinitely, and the organism can be said to be an autochthonous aquatic organism (Colwell and Spira, 1992). The conclusion that *V. cholerae* O1 can persist for long periods in water is supported by the observation that *V. cholerae* O1 of the same biotype, serotype, phage type and toxin profile has been isolated over several decades in locations such as the Gulf of Mexico (Blake *et al.*, 1983; Shandera *et al.*, 1983). Endemic focus has also been reported in Australia and Latin America (Wachsmuth *et al.*, 1994).

In the aquatic environment, a strong association between levels of zooplankton and incidence of *V. cholerae* has been observed (Huq *et al.*, 1983). Adhesion to chitin has been shown to influence strongly the ecology of *V. cholerae*. The organism is chitinolytic and its ability to digest chitin seems to play a role in its persistence in the environment (Dastidar and Narayanaswami, 1968; Colwell and Spira, 1992; Araujo *et al.*, 1996). Cholerae *V. cholerae* has also been reported to attach to the hindgut of crabs (Huq and Colwell, 1996), and it is noted that the hindgut of crustaceans is an extension of the exoskeleton and is lined with chitin. Based on studies in Bangladesh, Colwell and Spira (1992) concluded that seasonality of cholera may be explained in that primary transmission is controlled by environmental factors such as temperature, salinity, nutrient concentration and zooplankton blooms as well as by seasonal variation in seafood harvesting and consumption and by direct water contact. Studies in Bangladesh show that simple filtration of drinking-water through a sari cloth removed zooplankton, most phytoplankton and particulates with a size > 20 μ m and that it was effective in removing 99 percent of *V. cholerae* (Huq *et al.*, 1996). Deployment of this filtration procedure in 65 villages in Bangladesh with a population of about 133 000 individuals yielded a 48 percent reduction in cases of cholera

(Colwell *et al.*, 2003). From the foregoing, it can be concluded that choleraogenic *V. cholerae* is mainly found associated with plankton in the upper part of the water column.

V. cholerae occurs in waters with salinities between 0.2 and 20 ppt (Colwell and Spira, 1992). Hence, the organism is not associated with fish and shellfish caught in offshore marine waters. Shrimp are bottom-living organisms living in offshore waters, and this may explain the poor association between marine shrimp and choleraogenic *V. cholerae* O1 and O139. In fact, there are very few records of isolation of *V. cholerae* O1 and O139 from shrimp. Studies from Southeast Asia indicate absence of *V. cholerae* O1 from raw shrimp (Karunasagar *et al.*, 1990; Fonseka, 1990; Rattagool *et al.*, 1990; Karunasagar *et al.*, 1992). Several studies on shrimp farms in India indicated an absence of choleraogenic *V. cholerae* in shrimp culture ponds (Nayyar, Karunasagar and Karunasagar, 1995; Bhaskar *et al.*, 1998; Otta, Karunasagar and Karunasagar, 1999; Shetty, 1999; Darshan, 2000). Dalsgaard *et al.* (1995a) found that *V. cholerae* O1 was present in 2 percent (2/107) of water, sediment and shrimp samples collected from a major shrimp culture area in Southeast Asia. However, subsequent testing of the isolates indicated absence of the *ctx* genes in both the O1 strains (Dalsgaard *et al.*, 1995b). During the cholera epidemic in Peru, Carvajal *et al.* (1998) investigated the prevalence of *V. cholerae* in association with marine fish. Only 2 out of 450 samples of fish and shellfish tested yielded *V. cholerae* O1.

Growth and survival in seafoods: The optimum temperature for growth is 37 °C with a range of 10–43 °C (ICMSF, 1996). The pH optimum for growth is 7.6, and *V. cholerae* can grow in the pH range of 5.0–9.6. The ability to grow under alkaline conditions is utilized in standard isolation procedures when food samples are pre-enriched in alkaline peptone water, which has a pH of 8.6. The water activity optimum for growth is 0.984, and growth can occur between 0.970 and 0.998. *V. cholerae* can grow in the salt range of 0.1–4.0 percent NaCl, while the optimum is 0.5 percent NaCl. *V. cholerae* O1 is highly sensitive to acidic environments and is killed within minutes in gastric juice with pH < 2.4. Therefore, normochlorohydric individuals are less susceptible to attack by cholera provided that the food matrix does not protect the organisms. *V. cholerae* O1 is also highly sensitive to desiccation, indicating the necessity to use well-dried containers in product handling to minimize the transmission of cholera. This organism is also heat-sensitive with a D-value of 2.65 min at 60 °C (ICMSF, 1996). The pathogen survives refrigeration.

Kolvin and Roberts (1982) measured growth of *V. cholerae* O1 in raw and cooked seafood. No growth was observed in raw prawns, mussels and oysters, but growth occurred in cooked shellfish. Levels of 10¹⁰ cells/g were reported in cooked prawns and mussels stored at 37 °C. At 22 °C, there was a lag phase of 8 h for the Classical biotype and 4 h for the El Tor biotype. However, the results of the study done by Kolvin and Roberts (1982) have been questioned, as their reported densities of 10¹⁰ cells/g shrimp are difficult to obtain in laboratory broth cultures, even under optimal growth conditions.

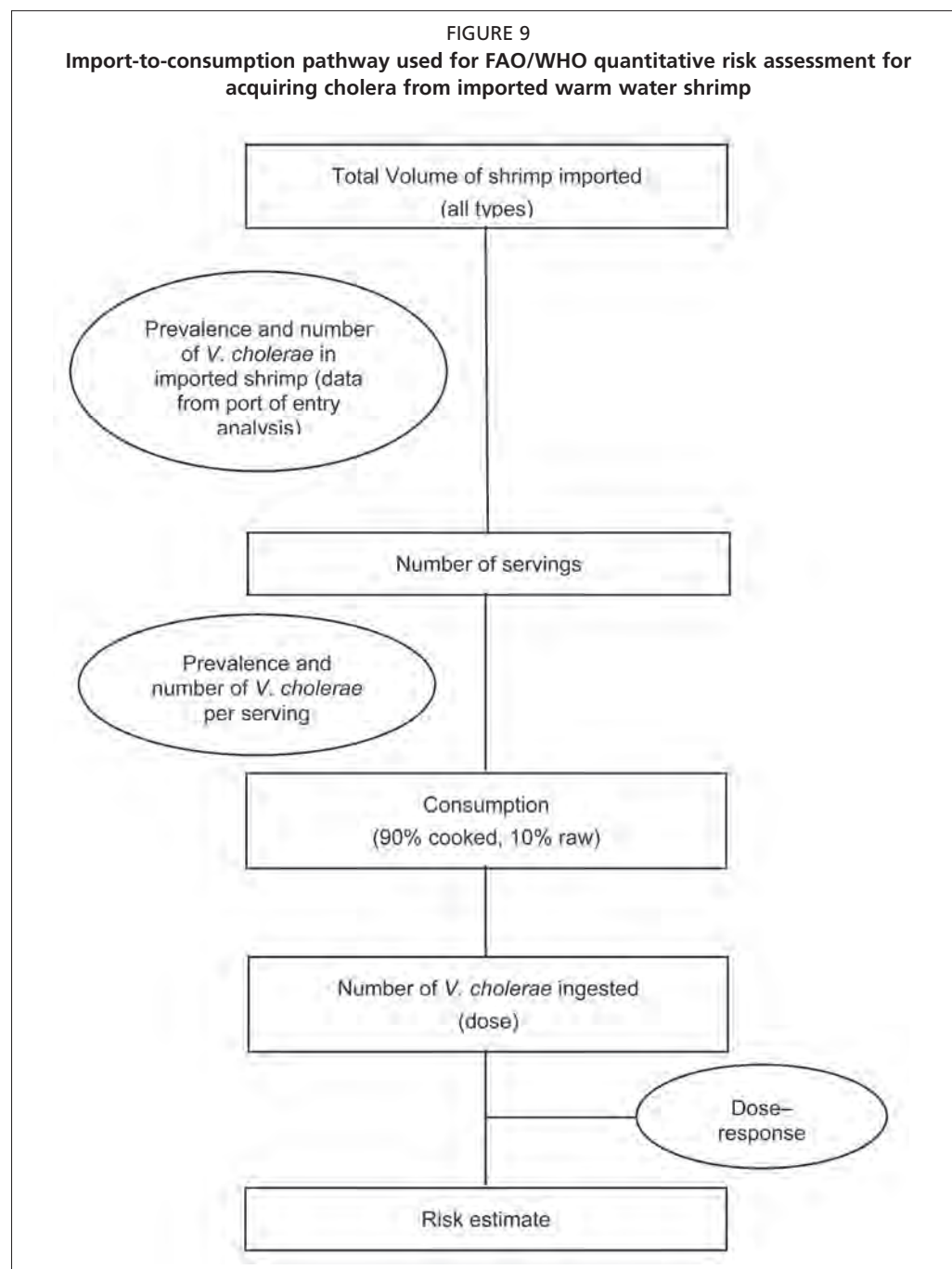
The literature on survival of *V. cholerae* O1 in foods indicates different patterns of decline and longevity during storage at refrigeration and freezing temperatures. Careful interpretation of results, as also recommended by ICMSF (1996), is required in order to account for methodological differences, including age of inoculums, preparation of food substratum, application of inoculums, enumeration procedure and medium. Most studies indicate that, while decline occurs, a proportion of the bacterial population remains viable. Starting with 10⁵/g *V. cholerae* O1 in raw shrimp, Pesigan, Plantilla and Rolda (1967) recorded viable cells after 4–9 days at 5–10 °C. Reilly and Hackney (1985) reported survival after 21 days at 7 °C from an initial density of 7.8 log/g. *V. cholerae* O1 inoculated at 10³–10⁴/g in ceviche, a marinated, ground or diced fish product, and, stored at 8 °C or 20 °C, it remained viable beyond the shelf-life of the product at both

temperatures (Torres-Vitela *et al.*, 2000). With respect to frozen storage, ICMSF (1996) reviewed literature from the 1930s that reported persistence for about 180 days and suggested that survival on fish was longer than on ground beef or vegetables. However, Nascimento *et al.* (1998) reported a 6-log reduction in shrimp in 30 days at -20°C . In this study, samples were inoculated by immersion in a *V. cholerae* O1 suspension for 5 min, followed immediately by freezing to -20°C . Survivors were enumerated by direct plating on thiosulphate-citrate-bile-sucrose (TCBS) with incubation at 35°C . Both the method of inoculation, with organisms located on a water film on the surface of shrimp, and recovery on a highly selective medium, could contribute to the observed rapid decline. A qualitative study, at temperatures above and below freezing, in which survivors were recovered by enrichment before plating on TCBS agar, and colonies confirmed by biochemical and serological testing, was reported by Corrales, Bainotti and Simonetta (1994). In fresh foods, including freshwater fish, *V. cholerae* O1 remained viable up to 90 days at -5°C and 30 days at -25°C . At non-freezing temperatures, survival time in fresh foods (milk, beef, fish and chicken) decreased with increasing temperatures: 7°C , 18–20 days; room temperature < 10 days; 35°C , < 2 days (Corrales, Bainotti and Simonetta, 1994). As the food samples had other bacteria, they spoiled rapidly at elevated temperatures, and spoilage organisms would have developed rapidly to the maximum population density supported by the product.

Risk assessment: FAO/WHO (2005b) explored the possibility of using the production-to-consumption pathway to assess the exposure to *V. cholerae* through consumption of warm water shrimp in international trade. Available literature indicates absence of *V. cholerae* O1/O139 in warm-water shrimp during primary production. In cholera endemic areas, asymptomatic carriers play an important role in the transmission of cholera. However, shrimp processed for export is handled under GHPs and the HACCP system. Therefore, personnel hygiene, quality of water and ice used for handling and processing are controlled under these conditions. Studies performed by DePaola *et al.* (1993) in Peru during the 1991 outbreak show that while *V. cholerae* O1 was present in all five samples of raw seafood collected from street vendors in Lima and Callao, it could be isolated from only 1/1 011 samples of seafood destined for export. This shows that even in an outbreak situation, it is possible to minimize contamination of seafood with choleraogenic *V. cholerae* by following GHPs and HACCP. Even when surface contamination takes place, some reduction in numbers occurs during handling and processing. Using artificially spiked shrimp, Dinesh (1991) showed that washing shrimp in tap-water brings about 1 log reduction in numbers. After harvest, shrimp are transported in ice, and a study conducted in India showed that storage of spiked shrimp in ice for 6 h led to a 3 log reduction in numbers (FAO/WHO, 2005b). Chilling and freezing would further cause reduction in numbers as discussed above. Shrimp processed for export may be frozen raw or after cooking. Cooking would further lead to a reduction in numbers of *V. cholerae*, if any, on shrimp. These provide evidence for the lack of involvement of internationally traded shrimp in outbreaks of cholera in shrimp-importing countries.

The FAO/WHO risk assessment also looked at the data from import testing laboratories in several shrimp-importing countries. Data for 21 857 samples of warm-water shrimp tested in Denmark, the United States of America and Japan showed that only two samples imported into Japan from India in 1995 were positive for *V. cholerae* O1. Implementation of the HACCP system was at an early stage in many shrimp-exporting countries in 1995. The levels of *V. cholerae* present were not known, as testing is normally done following enrichment of samples in broth. To perform a quantitative risk assessment, the import-to-consumption pathway (Figure 9) was used, and levels of *V. cholerae* in shrimp at import were statistically derived based on data that 2/21 857 samples were positive when 25 g each were enriched. The

serving size was estimated to be 275 g, and it was assumed that 10 percent of imported warm-water shrimp was consumed raw and 90 percent consumed after cooking.



Source: FAO/WHO (2005b).

Data from human volunteer studies conducted in the 1970s and 1980s were used to construct a dose-response curve. The estimate indicates that three out of every billion servings could result in cholera. However, epidemiological records show no documented case, and the low estimate obtained would be because two samples were positive in 1995. There has been no subsequent detection of choleraogenic *V. cholerae* at

port-of-entry testing laboratories. This confirms that the risk of transmitting cholera through warm-water shrimp in international trade is very low.

3.2.1.5 *Salmonella*

The genus *Salmonella* is a member of the family Enterobacteriaceae, and the taxonomy and nomenclature of the members of this genus have been the subject of considerable debate among specialists. Currently, two species are recognized (Tindall *et al.*, 2005): *Salmonella enterica* and *Salmonella bongori*. Six subspecies are recognized in *S. enterica* (Table 17). More than 2 500 serotypes have been recorded, of which the majority (59 percent) belong to *S. enterica* subsp. *enterica*, which are also responsible for 99 percent of *Salmonella* infections in humans and warm-blooded animals (Brenner *et al.*, 2000).

TABLE 17

***Salmonella* species and subspecies**

Species and subspecies	Number of serotypes	Usual habitat
<i>Salmonella enterica</i>		
subsp. <i>enterica</i>	1 504	Warm-blooded animals
subsp. <i>salamae</i>	502	Cold-blooded animals and environment (CBAE)
subsp. <i>arizonae</i>	95	CBAE
subsp. <i>diarizonae</i>	333	CBAE
subsp. <i>houtenae</i>	72	CBAE
subsp. <i>indica</i>	13	CBAE
<i>Salmonella bongori</i>	22	CBAE

Sources: Modified from Brenner *et al.* (2000) and Popoff, Bockemuhl and Gheesling (2004).

Each subspecies has several serovars defined by characteristic antigenic formulae, e.g. *S. enterica* serovar Typhi, *S. enterica* serovar Typhimurium, *S. enterica* serovar Enteritidis. The names may be abbreviated: *S. Typhimurium*, *S. Enteritidis*, etc. Serovars belonging to other subspecies are identified by antigenic formulae (D'Aoust, 2000; Popoff, Bockemuhl and Gheesling, 2004) and are not named. The antigenic formula indicates somatic (O) antigens and flagellar (H) antigens. Some salmonella serovars always express flagellar protein with the same antigenic specificity (e.g. Dublin, Enteritidis, Typhi) and such an H antigen is called monophasic. Most *Salmonella* serovars can produce flagella with two different sets of antigens, i.e. phase 1 and phase 2 antigens. The antigenic formula is written as follows: O antigens: Phase 1 H antigen(s): Phase 2 H antigen(s), e.g. an isolate with antigenic structure 4,5,12:i:2 is *S. Typhimurium*.

Public health outcomes: Human infections with *Salmonella* could lead to several clinical conditions such as typhoid fever (enteric fever), acute gastroenteritis or systemic non-typhoid infections. Enteric fever is caused by *S. Typhi* and *S. Paratyphi*, which are well adapted for invasion and survival in human tissues. The incubation period ranges from 7 to 28 days, and clinical manifestations include diarrhoea, prolonged spiking fever, abdominal pain, headache and prostration. The acute phase of the disease may be followed by a chronic carrier state. Improvement of hygiene and chlorination of drinking-water led to a rapid decline in the number of cases of typhoid fever in industrialized countries. However, occasional outbreaks have been reported (Valenciano *et al.*, 2000; Olsen *et al.*, 2003). In developing countries, typhoid fever is still a major problem, and the global disease burden in 2000 was estimated to be 2.16 million, with 216 000 deaths (Crump, Luby and Mintz, 2004). Non-typhoidal *Salmonella* constitute the largest cause of bacterial food-borne illness in developed countries. In Europe, in the period 1993–98, *Salmonella* (*S. Enteritidis* being the most common serovar, frequently linked to eggs) was involved in 126 303 cases

(18 159 outbreaks) accounting for 77.1 percent of outbreaks in which a causative agent was identified. The FoodNet data in the United States of America indicates that, in 2004, *Salmonella* was involved in 6 498/15 363 cases of food-borne illness caused by bacteria, and that *S. Typhimurium* followed by *S. Enteritidis* were the most common serovars involved (CDC, 2006). The involvement of different serovars in human infections globally is ranked in Table 18. Acute gastroenteritis caused by non-typhoidal *Salmonella* generally has an incubation period of 8–72 h and the clinical condition is generally self-limiting, although infection with some strains may degenerate into systemic infections and lead to various chronic conditions. Infection with serovars *S. Dublin* and *S. Choleraesuis* may lead to septicaemia. Supportive therapy such as fluid and electrolyte replacement is adequate for most uncomplicated cases, and antibiotic therapy may lead to prolonging the carrier state due to antibiotic-induced suppression of the native gut flora that normally competes with *Salmonella* (D'Aoust and Maurer, 2007). Antibiotic therapy is recommended only for patients who are severely ill and for those with risk factors for extra-intestinal spread of infection. Acute illness may be followed by a period of faecal shedding, which may last several weeks. In a review of 32 reports, the median duration of shedding was 5 weeks, with less than 1 percent becoming chronic carriers (Buchwald and Blaser, 1984). During convalescence, children may shed up to 10^6 – 10^7 bacteria per gram of faeces (Cruickshank and Humphrey, 1987). The infectious dose of *Salmonella* varies, with infants, elderly and immunocompromised individuals being more susceptible than healthy adults. Human volunteer studies indicate that a high number of cells (10^5 – 10^7 cells) are required to cause infection, but data from outbreak investigations suggest that low number of cells can cause infections (Kothary and Babu, 2001). The virulence of the serovars also varies, and, generally, low infectious dose (1–100 cells) is observed when ingested with foods with high fat content, e.g. chocolate, cheese or meat, and this has been attributed to the protection for *Salmonella* entrapped in hydrophobic lipid micelles against gastric acidity (D'Aoust and Maurer, 2007).

Although *Salmonella* is a major cause of food-borne illness, fish and fishery products are rarely involved. In the period 1988–1992, only 5 percent of *Salmonella* illnesses in the United States of America were due to seafood (Bean *et al.*, 1997). In New York, out of 273 outbreaks of food-borne salmonellosis in the period 1980–1994, only 4 were due to seafood (Wallace *et al.*, 1999). Outbreaks involving seafood have been reported from Japan. *S. Champaign* was involved in 330 cases in children who consumed cuttlefish that had been left to thaw at room temperature for 30 h and then boiled for a short period (Ogawa *et al.*, 1991). Contaminated well water of a squid processing plant in Japan was found to be the source of *Salmonella* that affected more than 400 people in 1999, and, in the same year, cuttlefish snacks contaminated with *S. Chester* was involved in an outbreak that affected more than 1 500 people (D'Aoust and Maurer, 2007). *S. Livingstone* was the cause of an outbreak that occurred in Norway and Sweden in 2001 in which fish gratin manufactured in Sweden was implicated and the egg powder ingredient in fish gratin was suspected to be the source (D'Aoust and Maurer, 2007). One outbreak in which 16 people became ill after a reception in a hotel in the United Kingdom of Great Britain and Northern Ireland in 1981 was attributed to frozen prawns (PHLSC, 1983). Although the implicated food was not tested, only those who ate prawns were affected, and *S. Bareilly* and *S. Hindmarsh* were isolated from the patients. It is not clear whether the prawns were prepared with any other ingredients, which could have been a source of *Salmonella*.

TABLE 18

Dominant *Salmonella* serotypes associated with human illness, and seafood/aquaculture environment

Human illness associated global rank 2002a ^a	Seafood associated rank occurrence 1990–98 ^b	Aquaculture environment (not rank ordered) 2001–03 ^c
Enteritidis (1)	Weltevreden (1)	Weltevreden
Typhimurium (2)	Senftenberg (2)	Paratyphi-B (predominantly biovar Java)
Newport (3)	Lexington (3)	Senftenberg
Heidelberg (4)	Paratyphi-B (4) (predominantly biovar Java)	Houten
Infantis (5)	Enteritidis (5)	Abaetetuba
Hadar (6)	Newport (6)	Derby
Virchow (7)	Thompson (7)	Aberdeen
Javiana (8)	Lanka (8)	Javiana
Saintpaul (9)	Virchow (9)	Hvittingfoss
Montevideo (10)	Hvittingfoss (10)	Give
Paratyphi B (16)	Typhimurium (12)	Newport
Weltevreden (20)	Derby(14)	

^a Galanis *et al.* (2006).^b Heinritz *et al.* (2000).^c Data from Hatha, Maqbool and Kumar (2003); Koonse *et al.* (2005); Kumar *et al.* (2009); Wan Norhana *et al.* (2010).

Source: FAO (2010a).

Association with aquatic environment: Although the normal habitat of *S. enterica* subspecies *enterica* is the gut of warm-blooded animals, very few serovars are host adapted and others may be found in the environment for long periods. The habitat for some of the subspecies is cold-blooded animals and environment (Table 17). *Salmonella* has been isolated from several aquatic environments in different parts of the world (Cherry *et al.*, 1972; Alonso *et al.*, 1992; Winfield and Groisman, 2003). Waterbodies contaminated with faecal matter from humans and animals (including birds) may contain this pathogen. *Salmonella* can survive in human waste for 10–15 days in septic systems and, through seepage from septic tanks, sewage and storm runoff, reach surface waters. It can survive and even multiply in estuarine waters depending on environmental conditions (Rhodes and Kator, 1998). *Salmonella* may colonize marine mammals such as killer whales, bottlenose dolphins, seals, sea lions, elephant seals and porpoises (Higgins, 2000; Old *et al.*, 2001; Fenwick *et al.*, 2004; Stoddard *et al.*, 2005), and the organisms shed by these mammals may contaminate other marine fish. In the period 1990–2002, 21.7 percent of harbour porpoises in England and Wales, the United Kingdom of Great Britain and Northern Ireland, were positive for *Salmonella*. On San Miguel Island, California, the United States of America, 33 percent of fur seal pups and 40 percent of sea lion pups were positive for *Salmonella* (Higgins, 2000).

Salmonella has been isolated from freshwater catfish ponds in the United States of America with a prevalence of 5 percent (Wyatt, Nickelson and Vanderzant, 1979), and from eel culture ponds in Japan with a prevalence of 21 percent (Saheki, Kobayashi and Kawanishi, 1989). The prevalence of *Salmonella* was found to be 16 percent in shrimp and 22.1 percent in mud/water in Southeast Asia (Reilly and Twiddy, 1992). In tropical shrimp aquaculture ponds, the risk of finding *Salmonella* was higher in ponds with high faecal coliform counts (Koonse *et al.*, 2005). However, in oysters from the United States of America, prevalence was related to season (13.4 percent positive in summer and 1.6 percent in winter) and the region, but did not correlate with faecal coliform levels (Brands *et al.*, 2005). Presence in trout farms in Spain (Gonzalez *et al.*, 1999) and long-term persistence of *Salmonella* in fish feed plants in Norway (Nesse *et al.*, 2003) has been reported. In the period 2000–04, 3.78 percent of environmental samples from Norwegian fish feed production facilities were positive for *Salmonella*. However, the serovars recovered were mostly *S. Senftenberg* and *S. Montevideo*,

which account for 2 percent of human cases in Norway (Lunestad *et al.*, 2007). In the period 1996–97, 574 isolates of *Salmonella* belonging to 41 serotypes were obtained from the Tech River (France), some serotypes being specific to flood events (Baudart *et al.*, 2000). A four-year study of coastal waters of Galicia, northwest Spain, showed a prevalence of 2.4 percent in molluscs and seawater, with *S. Senftenberg* being the most predominant (42 percent) among 20 different serotypes (Martinez-Urtaza *et al.*, 2004a). The presence of *S. Senftenberg* could not be correlated with environmental parameters, while the presence of other serotypes was associated with wind and rainfall events. *S. Senftenberg* has been very rarely reported in human infections and is halotolerant as it has been isolated from brines with a salt concentration of 30 percent (Martinez-Urtaza *et al.*, 2004b). *S. Senftenberg* has been isolated from mussel processing units in Spain, and processing units that did not use brine were negative for this organism (Martinez-Urtaza *et al.*, 2004b). In China, Hong Kong SAR, the *Salmonella* serovars found in coastal waters and shellfish were *S. Derby*, *S. Infantis* and *S. Anatum*, while the serovars isolated from clinical cases were *S. Typhimurium*, *S. Derby* and *S. Enteritidis* (Yam *et al.*, 2000).

This organism has been isolated from various fish and shellfish in markets in several countries. Analysis of 11 312 imported and 768 domestic seafood products in the United States of America in the period 1990–98 revealed that 10 percent of imported and 2.8 percent of domestic raw seafood was positive for *Salmonella*, and the overall incidence was 7.2 percent for imported and 1.3 percent for domestic seafood (Heinitz *et al.*, 2000). The most frequent serotypes in imported seafood were *S. Weltevreden*, *S. Senftenberg*, *S. Lexington* and *S. Paratyphi B*. These most common serotypes have rarely (<0.5 percent) been observed in human illness in the United States of America (Helrick *et al.*, 1997). *S. Enteritidis* ranked fifth and *S. Typhimurium* ranked twelfth (Heinitz *et al.*, 2000). *S. Weltevreden* was also the most common serotype isolated from imported food (including seafood) in the United States of America in 2000 (24/187) followed by *S. Thompson* (13/187), *S. Lexington* (12/187), and a number of other serotypes (Zhao *et al.*, 2003). Also in the period 2001–05, *S. Weltevreden* was the most predominant serotype and PFGE analysis indicated genetic diversity in the 37 isolates of this serotype (Ponce *et al.*, 2008). Analysis of shellfish from authorized harvesting beds in the United Kingdom of Great Britain and Northern Ireland indicated 8 percent positive for *Salmonella* and 2 percent were molluscs from beds classified as Category A (Wilson and Moore, 1996). Heinitz and Johnson (1998) reported a 3.2 percent incidence in 156 smoked fish. In Malaysia, 25 percent of raw prawns on the market were positive, the serovars found being *S. Blockley*, *S. Weltevreden* and *S. Agona* (Armugaswamy *et al.*, 1995); and in India, 1 percent of the 500 market prawns tested were positive, the serovars being *S. Newport* and *S. Infantis* (Prasad and Pandurangarao, 1995). In oysters from the United States of America, *S. Newport* was the predominant serovar (Brands *et al.*, 2005). In Thailand, *S. Weltevreden* accounted for 26 percent of the isolates from seafood (Bangtrakulnonth *et al.*, 2004). In a study of 353 imported seafood items in Japan, 2/47 black tiger shrimp were positive, both with *S. Weltevreden*, and the contamination level in seafoods was < 30–40 MPN/100 g (Asai *et al.*, 2007). Also in Japan, *S. Enteritidis* is the most common serovar involved in human infections, accounting for 62 percent in 2002 and 2003, 47 percent in 2004 and 50 percent in 2005 (IDSC, 2006). In Norway, *S. Typhimurium* and *S. Enteritidis* account for 70 percent of human salmonellosis cases (Lunestad *et al.*, 2007). Association of *Salmonella* with seafood and the aquaculture environment is indicated in Table 18.

Recent molecular studies indicate that, within a serotype, clinical and animal strains may be distinct (Heithoff *et al.*, 2008). While all *S. Typhimurium* from animal clinical cases were virulent in mice, only 16/41 human isolates showed this ability. Many (10/29) human gastroenteritis isolates did not have the virulence plasmid found with

all animal clinical isolates. This suggests that it may be possible to differentiate human and animal pathogenic strains.

Factors affecting survival and growth in foods: *Salmonella* is a mesophilic organism and the growth rate of this organism is markedly reduced at temperatures < 15 °C while the growth of most strains is prevented at < 7 °C (ICMSF, 1996). Most studies on minimum growth temperature have been done with beef, chicken or eggs using serovars such as Typhimurium or Enteritidis common in these foods. However, these are not common serotypes in seafoods. In raw seafoods containing a variety of bacteria, *Salmonella*, where present, has to compete with other flora for growth. *S. Heidelberg* had a generation time of 28 h and 31 h in the fish English sole and sterile crab respectively at 8 °C (ICMSF, 1996). In cooked crab inoculated with *Salmonella* and stored at 8–11 °C under modified atmospheres containing low levels of CO₂ (20–50 percent), proliferation of *Salmonella* has been reported (Ingham, Alford and McCown, 1990). *Salmonella* have ability to proliferate at pH values ranging from 3.8 to 9.5, with the optimum being 7.0–7.5 (ICMSF, 1996). Growth of *Salmonella* is generally inhibited at 3–4 percent NaCl, but salt tolerance increases with increasing temperature in the range 10–30 °C (D'Aoust and Maurer, 2007) and minimum water activity for growth is 0.94 (ICMSF, 1996). Although the resistance of *Salmonella* to drying varies, this organism may survive for months or even years in dried products and has been frequently isolated from fishmeal, meat and bone meal, maize and soy products (Lunestad *et al.*, 2007). A decrease in *Salmonella* numbers occurs during freezing and frozen storage, but this process does not guarantee elimination of salmonellae in foods (ICMSF, 1996). *Salmonella* are heat-sensitive and D-values are influenced by the water activity, nature of the solutes and pH of the suspending medium (ICMSF, 1996). Typical D-values reported for *Salmonella* are 0.176 min in chicken at 70 °C, and 0.36 min in ground beef at 63 °C (FAO/WHO, 2002). Some strains of *Salmonella* such as *S. Senftenberg* 775W may show higher heat resistance (ICMSF, 1996). *S. Senftenberg* is the serovar often isolated from fish feed (Lunestad *et al.*, 2007).

Risk assessment and management: FAO/WHO expert groups have considered the public health risk due to *Salmonella* in aquaculture (FAO, 2010a) and in live bivalve molluscs (FAO/WHO, 2011c). Epidemiological links between *Salmonella* and products of aquaculture are very low (Table 19).

TABLE 19
Seafood-associated outbreaks in European Union (Member Organization) (2007) and in the United States of America (1998–2002)

Food vehicle	Number of outbreaks	Number of <i>Salmonella</i> outbreaks	% of outbreaks associated with <i>Salmonella</i>
European Union (Member Organization)			
Fish and fishery products	130	3	2.3
Crustaceans, shellfish and molluscs	75	2	2.7
All food vehicles	2 025	590	29.1
United States of America			
Fish	337	4	1.1
Shellfish	151	2	1.3
All food vehicles	6 647	585	8.8

Source: FAO (2010a).

There are a variety of pathways reported as to how *Salmonella* can enter aquaculture environments, ranging from wild animals to domestic stock and poor sanitation. Control of such pathways, such as land runoff during rains and control of wild animals

in the farm environment, could pose a major challenge. Good hygiene practices during aquaculture production and biosecurity measures can minimize but not fully eliminate *Salmonella* in products of aquaculture.

3.2.1.6 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, motile bacterium. It is very common in environments that are wet or moist and contain organic nutrients, including soil and decaying vegetation, but also in many food processing environments. It is also commonly found in the faeces of healthy birds and mammals, including humans. It is a common contaminant of fresh, or lightly preserved, foods. In addition to a form of gastroenteritis, infection by *L. monocytogenes* can cause a rare but life-threatening systemic, food-borne disease called listeriosis.

Despite the relative ubiquity of *L. monocytogenes*, listeriosis is a rare infection, and the systemic illness predominantly affects people with reduced immune function, including pregnant women, the elderly (> 60 years old), foetuses and neonates (up to 30 days), those with diseases or receiving medication that reduces immune function (e.g. HIV/AIDS, diabetes, alcoholism, organ transplant recipients, patients undergoing cancer therapies, and those with autoimmune disease) or those taking antacids.

In rare cases, *L. monocytogenes* can be transmitted from infected people to others or from infected animals to humans. However, listeriosis is considered to result predominantly from consumption of foods contaminated by *L. monocytogenes*, and, in particular, perishable, RTE foods with extended shelf-lives, i.e. those that do not require cooking before eating but would normally require refrigeration. Many lightly preserved types of seafood are in this category, and *L. monocytogenes* is considered to be a risk for consumers of those foods.

***Listeria monocytogenes* as a food-borne pathogen:** *L. monocytogenes* is one of seven species in the genus *Listeria*, the seventh species (*L. marthii*) having been described in 2010 (Graves *et al.*, 2010). Of the known species, only *L. monocytogenes* is considered a pathogen to human, although a few cases of infection from *L. grayi*, *L. seeligeri* and *L. ivanovii* have been reported (Rocourt *et al.*, 1986; Rapose, Lick and Ismail, 2008; Guillet *et al.*, 2010). Listeriae are closely related to lactic acid bacteria and, in many foods, lactic acid bacteria compete with *Listeria monocytogenes* and can suppress their growth (Leroi, 2010).

Among pathogens affecting humans and other mammals, *L. monocytogenes* has an unusual tolerance of low temperature with most strains able to grow at 4 °C and some strains being reported to grow at temperatures as low as 0 or even –2 °C. It also has a relatively high salt tolerance and is able to grow in 10–12 percent (w/w) salt (corresponding to water activity of ~0.92). It grows almost equally well in anaerobic environments as in air. Its pH range for growth is not unusual and is, approximately, pH 4.3–9.6. *L. monocytogenes* is readily eliminated by normal cooking but, because of its relative ubiquity, can re-contaminate cooked foods after processing if they are not protected by packaging. Typically, such contamination is at low levels, e.g. a few to tens of cells per gram, even at the point of sale (Jørgensen and Huss, 1998; Gombas *et al.*, 2003; Little *et al.*, 2009). Available evidence suggests that high doses are usually required to initiate infection, even among the immunocompromised population, so that limiting or preventing the growth of *L. monocytogenes* in foods will be an important risk management strategy.

The above characteristics make *L. monocytogenes* a potential hazard in perishable RTE foods with extended shelf-lives (e.g. weeks or months) achieved by refrigeration and/or mild preservation methods, including salt, smoke, fermentation, vacuum-packaging, and modified atmosphere packaging, but which may not completely prevent the growth of the organism. This applies to a range of seafood products, including marinated muscles, prawns, pasteurized crustacea, and smoked fish products.

Cold-smoked products in particular have received much attention in this regard (FDA/USDA/CDC, 2003; FAO/WHO, 2004a; Pouillot *et al.*, 2007; Pouillot *et al.*, 2009) because of a high prevalence of detection of *L. monocytogenes* in such foods and persistent of contamination fish processing plants.

Epidemiological evidence suggests that listeriosis has been associated with consumption of shrimps (Riedo *et al.*, 1994), smoked mussels (Brett, Short and McLauchlin, 1998; Misrachi, Watson and Coleman, 1991), “gravad” trout (Ericsson *et al.*, 1997), and smoked trout (Miettinen *et al.*, 1999). In addition, Aureli *et al.* (2000) described an outbreak involving corn and tuna salad. However, many of these outbreaks involved the gastrointestinal form of the disease and, despite the interest in RTE smoked fish as a source of listeriosis, there are very few documented cases of systemic listeriosis due to seafoods.

Listeriosis: Historically, listeriosis was considered to be characterized by an invasive infection, often leading to septicaemia with or without infections of the central nervous system such as meningitis, meningoencephalitis, rhomboencephalitis or brain abscess. In the case of pregnant women, while the mother will often experience mild flu-like symptoms, her foetus may be stillborn, aborted or be born with generalized infections. Less common symptoms include localized infections such as endocarditis, peritonitis and arthritis. Skin infections may also occur in some patients.

The incubation period is variable, ranging from 3 to 70 days, and, as most people do not remember their food consumption from months earlier, it is often difficult to trace the food that was the source of the pathogen. The median incubation period is approximately three weeks. If diagnosed, the disease can usually be treated effectively with a range of common antibiotics. The mini-review by Drevets and Bronze (2008) provides a summary of the various syndromes.

Miettinen *et al.* (1999) documented that *L. monocytogenes* may also cause a non-invasive febrile gastroenteritis in otherwise healthy people. An outbreak of gastrointestinal illness from a tuna and corn salad, affecting > 1 500 schoolchildren and adults in Italy, established the existence of a febrile gastroenteritis form of listeriosis, and this is now a recognized syndrome (Drevets and Bronze, 2008; Alleberger and Wagner, 2009). The incubation period for this form of the disease ranges from 6 to 50 h, and symptoms usually resolve without treatment after one or two days. Symptoms are described as “mild flu-like”, including diarrhoea, abdominal pain, fever, muscle pain and headaches. Ooi and Lorber (2005) summarize the outbreaks to that time and provide more detail of this form of the disease.

Epidemiology of listeriosis: Despite the prevalence of *Listeria monocytogenes* in foods and in natural and food processing environments, and its asymptomatic carriage in 5–10 percent of humans and domestic animals, listeriosis is a rare disease. In developed nations, the incidence is typically in the range of 0.3–1.3 cases per 100 000 people per year, with median levels of from ~0.3/100 000 to 0.5/100 000. The rates observed do not seem to correlate with different regulatory systems and control programmes implemented in various nations (Todd and Notermans, forthcoming). As noted above, certain groups in the population are at much greater risk of invasive infection. Table 20 indicates the relative susceptibility of people with known predisposing factors for listeriosis. Importantly, the fatality rate among those that develop invasive infection is very high and, in outbreaks, ranges from 20 to 40 percent of cases.

The epidemiology of listeriosis has changed in many European States from about 2000 to the time of writing (2011), with incidence rates increasing by from twofold to threefold in many countries (Goulet *et al.*, 2008; Allerberger and Wagner, 2009), and with a much higher proportion of cases occurring in the elderly population. At the same time, the infections observed have been increasingly bacteraemia but without central nervous system infection. However, in the United States of America, the incidence has remained relatively constant over the same period, as it did in Canada

from 1995 to 2004 (Clark *et al.*, 2010). In Australia, the incidence rate also remained relatively constant from 1995 to 2010 (CDNA, 2011) but the relative incidence for pregnant women and/or perinates decreased, probably due to aggressive education campaigns about listeriosis risks aimed at pregnant women (Torvaldsen *et al.*, 1999; Bondarianzadeh, Yeatman and Condon-Paoloni, 2007) while the incidence in the elderly population increased. Several epidemiological studies have attempted to discern the reasons for the upsurge in Europe (Swaminathan and Gerner-Smidt, 2007; Warriner and Namvar, 2009; Gillespie *et al.*, 2010a, 2010b; Khatamzas *et al.*, 2010) but, at the time of writing, there no clear explanation has been presented.

TABLE 20
Relative susceptibility to listeriosis for subpopulations with known predisposing conditions

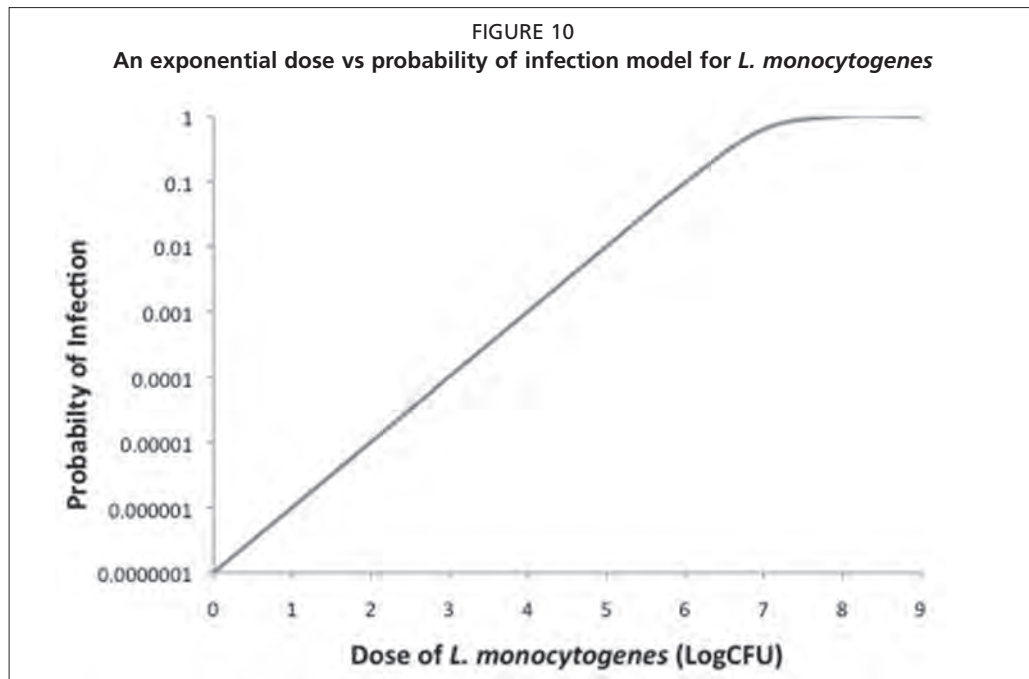
Condition	Relative susceptibility
Transplant	2 584
Cancer – blood	1 364
AIDS	865
Dialysis	476
Cancer – pulmonary	229
Cancer – gastrointestinal and liver	211
Non-cancer liver disease	143
Cancer – bladder and prostate	112
Cancer – gynaecological	66
Diabetes, insulin dependent	30
Diabetes, non-insulin dependent	25
Alcoholism	18
More than 65 years old	7.5
Less than 65 years, no other condition	1

Source: Reproduced from FAO/WHO (2004a).

Dose vs probability of invasive infections in listeriosis: Risk assessment and animal model studies (FDA/USDA/CDC, 2003; Chen *et al.*, 2003; FAO/WHO, 2004a; Williams *et al.*, 2009) suggest that the ID₅₀ (dose required to cause infection in 50 percent of cases) for *L. monocytogenes* is millions of cells, even among the immunocompromised population. Wide variability in ID₅₀ is inferred from animal studies, however, ranging over seven orders of magnitude (see Table 2.11 in FAO/WHO, 2004a). The relative susceptibility of humans (Table 20) indicates that susceptibility ranges over three orders of magnitude. Taken together, it can be expected that the “infectious dose” could vary enormously depending on the strain involved and human population exposed, and estimates of infectious doses estimated from outbreaks in human populations (summarized in FAO/WHO, 2004a) range over five to six orders of magnitude, supporting the above inference.

According to FAO/WHO (2003b) the most credible model that relates dose ingested to the likelihood of an infection is the “exponential” model, which assumes that each cell has an equal probability of causing infection, and that each cell ingested acts independently. This means that there is no threshold dose and that the probability of infection is simply proportional to the dose up to some dose beyond which infection is virtually inevitable (i.e. the probability of infection cannot increase further). Figure 10 is an example of an exponential dose–response model.

In Figure 10, the dose and probability are both plotted on logarithmic scales, leading to a straight line below the asymptotic value, as is expected by the assumptions of the model. Many such plots of dose vs probability of infection appear sigmoidal, incorrectly suggesting a threshold dose, because they plot log dose versus arithmetic probability.



Source: Based on estimates in Williams *et al.* (2009).

Dose–response models for *L. monocytogenes* inferred from epidemiological data and estimates of total food-borne exposure (Buchanan *et al.*, 1997; FDA/USDA/CDC, 2003; FAO/WHO, 2004a) have generated ID₅₀ estimates for immunocompromised people of $> 10^{10}$ cells, even for an “average” immunocompromised person. Williams *et al.* (2009) challenged such findings, using data for pregnant primates and guinea pigs (considered to be an appropriate animal model for human listeriosis because they also have the E-cadherin protein involved in initial infection by *L. monocytogenes*), which show that the ID₅₀ for abortion is $\sim 10^7$ cells. However, in those studies, known virulent strains were used, and the difference in ID₅₀ estimates may reflect the specific circumstances of their estimation and assumptions made. However, it does highlight the variability in virulence observed among strains of *L. monocytogenes*.

Tracing and identifying strains of *Listeria monocytogenes*: Given the wide differences in virulence among strains of *L. monocytogenes* and its relatively common occurrence in foods and food processing environments, there has been much interest in finding easily determined markers of virulence in *L. monocytogenes* that might be used to better evaluate and manage the risk of *L. monocytogenes* in foods, e.g. that there might be some tolerance of strains of low virulence in foods that did not support extensive *L. monocytogenes* growth. Equally, there is great interest in understanding the source (or sources) of *Listeria monocytogenes* in foods and food processing plants, and their relationship to strains involved in human and animal disease, and for recognizing and resolving outbreaks. Thus, to manage the risk of *L. monocytogenes* in foods, it is necessary to be able to differentiate strains.

The first typing scheme for *L. monocytogenes* involved somatic (O) and flagellar (H) antigens and divided differentiates the species into 13 serovars (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 6a and 6b). Most isolates involved in human disease belong to the serotypes 1/2a, 1/2b, 1/2c and 4b.

Serotyping has not provided the ability to confidently discern “important” strains, nor the needed discrimination for epidemiological investigations. Ongoing research has resulted in the recognition of four evolutionary “lineages” of *L. monocytogenes* that differ in their correlation with human and animal illness (Ragon *et al.*, 2008; Orsi, den Bakker and Wiedmann, 2010). Strains of lineage I and lineage II are most

often isolated from foods but strains of lineage I are more often involved in human food-borne listeriosis. Lineage III and IV strains are rare and more often involved in animal disease but rarely isolated from foods or human cases. Lineage I includes the serotypes 1/2b, 4b and 4d, 4e and 3b while lineage II includes serotypes 1/2a, 1/2c, 3a and 3c. On the basis of cell-to-cell spread in *in vitro* cell culture assays, lineage I strains appear to have greater pathogenic potential. Lineage III strains include serotypes 4a, 4c and some 4b strains that differ from the 4b strains in lineage I. While lineage II strains are over-represented in foods, this may be an artefact of the use of certain selective media, which favours their recovery compared with other strains. In addition, lineage II strains are more resistant to bacteriocins and, in many foods in which *L. monocytogenes* is considered to present a risk, lactic acid bacteria are also present. This may also contribute to the observed prevalence of lineage II strains in foods. Conversely, a high proportion of lineage II strains have mutations in the protein internalin A, which reduces their ability to cause human infection. (Internalin A is key protein involved in the initial attachment and invasion of *L. monocytogenes* into a host cell; most strains in lineage I have intact internalin A). The abundance of strains with such mutations among lineage II may explain the apparent discrepancy between the abundance of *L. monocytogenes* in foods and the observed number of cases of listeriosis. However, internalin A is not the only protein involved in cell invasion and virulence, and lineage II strains can also cause sporadic human cases. In summary, both lineage I and lineage II strains are found in foods and, while lineage I is known to comprise more virulent strains, these correlations are not absolute and there is still no reliable means of discriminating “high risk” from “low risk” strains. Research to better understand these correlations is under way, and methods for delineating *L. monocytogenes* strains of increased virulence are ongoing. In terms of strain differentiation, PFGE has been widely used for surveillance and epidemiological investigations, but it is difficult to standardize between laboratories. Numerous other DNA-sequence-based methods are being developed and trialled. Zunabovic, Domig and Kneifel (2011) described and reviewed these methods in terms of their technological and scientific basis and their relevance for different practical applications.

Prevalence in fish and fishery products: *L. monocytogenes* is indigenous to terrestrial environments, where it is readily isolated from soil and decaying plant material. However, it is not typical of aquatic and marine environments, and the organism is not usually isolated from free open waters or from fish caught or cultured in such waters. In contrast, water close to agricultural runoff harbours the organism and, in principle, the bacterium must be assumed to be present, albeit in low levels, on raw fish (Gram, 2001; Huss, Ben Embarek and Jeppesen, 1995). Surveys of fresh or frozen finfish, and of filter feeding shellfish, summarized by Jinneman, Wekell and Eklund (2007) support these general conclusions.

However, for RTE seafoods in the data summarized by Jinneman, Wekell and Eklund (2007) the situation is different. From more than 20 surveys involving more than 45 groups of RTE seafood products (based on product and/or region of origin), the average contamination frequency was ~16 percent (SD = ~18 percent), and the median contamination rate was ~9 percent. More recent data not included in Jinneman, Wekell and Eklund (2007) include the results of: Gombas *et al.* (2003), based on foods purchased at retail outlets in the United States of America; Garrido, Vitas and Garcia-Jalon (2009), based on foods from markets in northern Spain; and Wagner *et al.* (2007) based on samples from retail outlets and private homes in Vienna, Austria. Wagner *et al.* (2007) found 19.4 percent (of 93 samples) of RTE fish and seafood at retail were positive for *L. monocytogenes*, while Gombas *et al.* (2003) reported 4.3 percent of 2 644 smoked seafoods and 4.7 percent of “seafood salads” positive for *L. monocytogenes*. Garrido, Vitas and Garcia-Jalon (2009) found

25 percent of samples of RTE smoked fish contaminated with *L. monocytogenes* with up to 60 percent prevalence in some brands.

As noted above, there has been much interest in the potential for cold-smoked fish products to cause listeriosis, which probably results from: (i) the absence of a listericidal step in the process of cold-smoking of fish; (ii) the fact that the product supports the growth of *L. monocytogenes* and has a relatively long refrigerated shelf-life (3–4 weeks); (iii) the fact that the product has a relatively large market and is traded internationally (FAO, 1999); and (iv) the fact that many surveys indicate that *L. monocytogenes* prevalence on the product is high, with rates of contamination of up to ~80 percent observed in cold-smoked fish products (Table 21). These circumstances have led to much research concerning the sources and ecology of *L. monocytogenes* in fish processing plants.

Listeria monocytogenes is isolated at much higher frequency from RTE seafood products than from raw materials. A number of studies on smoked-fish processing plants in the 1990s and early 2000s (see Hansen, Vogel and Gram, 2006) demonstrated that the processing environment is an important niche for *L. monocytogenes*. While some studies have found the same strains in both raw fish and finished products (Miettenan and Wirtanen, 2006), it is widely concluded that most contamination arises from strains that have colonized the factory, while raw material is only rarely a direct source of product contamination (Lappi *et al.*, 2004; Timothe *et al.*, 2004). More recent studies (e.g. Klaeboe *et al.*, 2006; Cruz *et al.*, 2008; Dass *et al.*, 2010; Chen *et al.*, 2010) have not changed the view that colonization of fish processing plants occurs and is the main source of contamination of RTE seafood products. The original source of the *L. monocytogenes* that come to colonize the factory may be raw fish, but it appears that conditions in the factory select for strains more able to colonize factories than others. For example, using DNA-typing methods, Wuff *et al.* (2006) found that similar strains colonized different processing plants, and that they persisted in some plants for years. Numerous studies have been conducted in fish processing plants to determine the sites of colonization and sites of transfer to RTE fish products. Brines used in preparation of smoked fish can harbour *L. monocytogenes*, particularly if they become diluted over time, as can hard-to-clean equipment such as slicers. Packaging areas have also been implicated.

Table 21 also shows that *L. monocytogenes* is commonly detected in heat-processed products subjected to a listericidal process, particularly those that involve extensive handling. Post-process contamination is the probable cause of this contamination. Cleaning and disinfection may temporarily remove the organism, which is often found in more permanent niches in the processing environment such as in drains or under floor mats.

Growth and survival in fish and fishery products: Jinneman, Wekell and Eklund (2007) provide a review of studies of the growth potential of *L. monocytogenes* in RTE seafoods, noting that seafood provides an excellent substrate for growth. In lightly preserved seafoods, a number of hurdles are employed to increase the shelf-life of the product, often in combination, including refrigeration, salt, phenolic (smoke) compounds, acidification with organic acids including lactate, acetate, sorbate, benzoate, citrate, or addition of salts of organic acids, addition of nitrite and modified atmosphere packaging including CO₂.

TABLE 21

Representative prevalence of *Listeria monocytogenes* in RTE seafood products

Product	No. of samples	% positive for <i>L. monocytogenes</i>
Blue crab (United States of America)	126	7.9
Fresh shrimp (Japan)	74	1.4
Fresh shrimp (Brazil)	178	17
Shrimp (multiple countries)	287	1.5
Fish (fresh, India)	51	0
Fish (fresh, Japan)	382	2.4
Fish (fresh, Trinidad)	61	2
Ceviche (Peru)	32	9
Cold-smoked salmon (Australia)	285	< 1
Cold-smoked salmon (Denmark)	340	20.9
Cold-smoked salmon (Switzerland)	100	24
Cold-smoked fish (Switzerland)	434	11.3
Cold-smoked fish (United States of America)	61	78.7
Gravad/smoked salmon or trout (Sweden, 6 data sets 1993–6)	344	14 (range 4–23)
Hot-smoked fish (Switzerland)	691	8.4
RTE seafood at retail (Austria)	93	19.4
RTE seafood at retail (United States of America)	2 644	4.3
Seafood Salads (United States of America)	2 446	4.7
Seafood salads (Iceland)	37	16

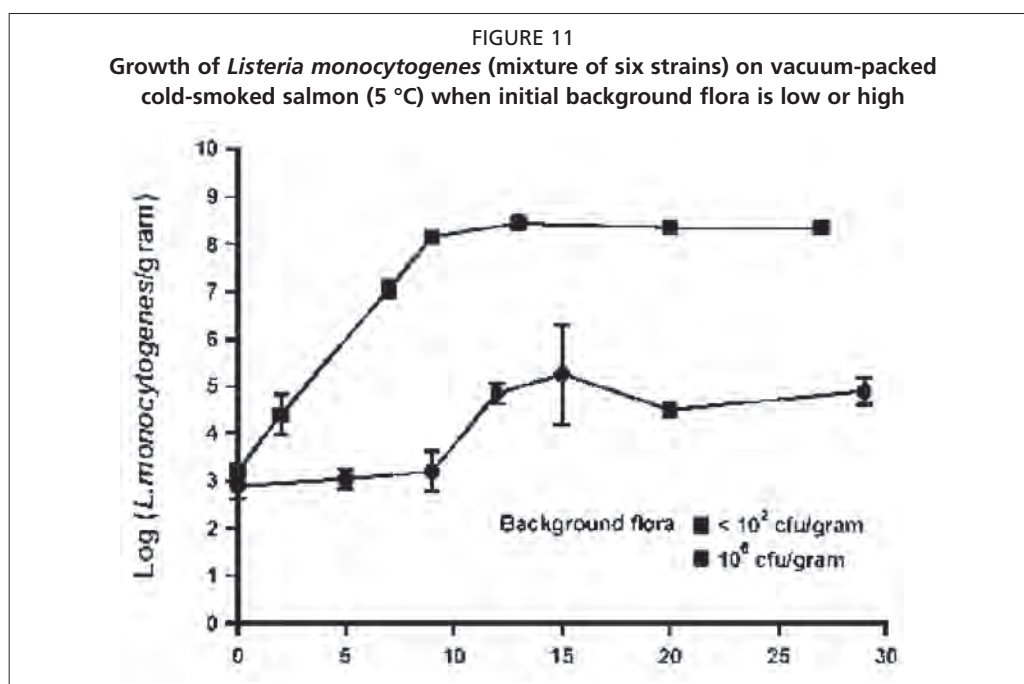
Source: Modified from Jinneman, Wekell and Eklund (2007) and supplemented.

The effects of these hurdles alone or in combination are conveniently summarized in a range of mathematical models. The most comprehensive of these models, and the one most extensively evaluated, is that of Mejlholm and Dalgaard (2007), which predicts the growth rate and limits to growth of *L. monocytogenes* in response to most of the hurdles listed above. The evaluation of that model, and several others, against 1 014 growth rate / growth limits datasets for *L. monocytogenes* in RTE foods, including 194 RTE seafood products, was presented by Mejlholm and Dalgaard (2009). The model evaluations undertaken showed that models can predict growth responses accurately for RTE products without added antimicrobials as well as for those with added salt, nitrite, organic acids and smoke components, and packaging atmospheres enriched with CO₂. However, the authors concluded that reliable predictions of growth rate are obtained when the model contains terms to account for the effects of all hurdles to *L. monocytogenes* growth that are present in the foods of interest. An expanded form of the model of Mejlholm and Dalgaard (2007), including the effects of all of the parameters described above, was subsequently developed and presented by Mejlholm and Dalgaard (2009). User-friendly software that embodies that model can also be accessed on a web page of the Danish Technical University (<http://sssp.dtuqua.dk/>).

Many of the data used in the evaluation referred to above involved foods deliberately inoculated with *L. monocytogenes*, but several authors have noted that growth in foods that are “naturally” contaminated (i.e. during production) with *L. monocytogenes* often appears much slower than that predicted by models based on artificially inoculated product samples. This discrepancy may be due to sublethal injuries during processing or may be partly explained by the so-called “Jameson effect” (Ross, Dalgaard and Tienungoon, 2000), and also known as “hidden fermentation” (Stiles, 1996) when caused by lactic acid bacteria, where the presence of a competitive microbiota in the food can depress the maximum cell density of other bacteria, including *L. monocytogenes*. Figure 11 shows an example of the effect.

The mechanistic basis of the Jameson effect is unknown, but it may be due to competition for space, production of toxic end products of metabolism, production of specific inhibitory compounds such as bacteriocins, production of

quorum-sensing compounds and “crosstalk” among related species, etc. Several studies have considered the interaction between lactic acid bacteria and other organisms, including *L. monocytogenes*, in foods, and have developed mathematical models to describe those interactions. The modelling software referred to above and available from the Danish Technical University also includes models for the effects of interactions between lactic acid bacteria and *L. monocytogenes*. The modelling approach itself is described in Giménez and Dalgaard (2004).



Source: From Huss, Jørgensen and Fønnesbech Vogel (2000).

The inhibition of *L. monocytogenes* growth in the presence of high levels of background microbiota evident in Figure 11 can be used deliberately as a preservation technology by adding a “bio-protective” competitive lactic acid bacterial flora that inhibits *L. monocytogenes* (Nilsson, Gram and Huss, 1999; Leroi, 2010). It is preferable to use homofermentative strains, which have lower spoilage potential. Even non-bacteriocin-producing lactic acid bacteria can induce the Jameson effect (Tome, Teixeira and Gibbs, 2006; Mellefont, McMeekin and Ross, 2008).

Traditionally, the only hurdles to growth of *L. monocytogenes* in smoked fish were salt (usually at 2–3 percent in the aqueous phase and leading to a water activity of 0.97–0.98) and phenolic compounds from smoke. The pH of the product is typically about pH 6.2, which has a minimal effect on the growth rate of *L. monocytogenes*, and there is no listericidal step in the process. Lactic acid bacteria are usually present and the product is usually vacuum-packed and refrigerated. Under these conditions, growth of *L. monocytogenes* is predicted (by many models) to occur and is also observed in practice. The doubling time of *L. monocytogenes* under these conditions is typically in the range 30–50 h. Given that the specified shelf-life of the product is often 3–4 weeks at refrigeration temperatures, there is potential for up to 3 000–5 000 000-fold increases in *L. monocytogenes* levels in the product prior to consumption, but this potential is often limited by the presence of competitive microbiota.

Given this potential growth and also the difficulty of preventing ad hoc contamination of the product, there has been increasing interest in the use of additional hurdles to *L. monocytogenes* growth in RTE foods, including seafood products. Experimental

treatments have included the addition of protective cultures, bacteriocins and bacteriocin-containing formulations, and addition of salts of organic acids such as sodium or potassium lactic acid or sodium diacetate, or both. While salts of lactic acid are relatively benign in terms of sensory changes, acetic acid and its salts are sensorially detectable above concentrations of ~0.25 percent (e.g. of Na-diacetate) but acetate is apparently more effective on a molar basis, possibly because it has a lower pK_a . Salts of organic acids are preferred because they do not greatly alter the pH of the products, although reduction of pH greatly enhances the antimicrobial activity of organic acids because the undissociated form of organic acids is typically hundreds of times more inhibitory to microbial growth than the dissociated form. In other lightly preserved RTE seafoods, other organic acids may also be used to minimize the potential growth of *L. monocytogenes* in the product.

Passive inactivation of *Listeria*: As a “rule of thumb”, when pathogens are prevented from growth by environmental hurdles, they are inactivated. Even when temperature *per se* is not lethal, the rate of inactivation is strongly affected by temperature (Zhang, Ross and Bowman, 2010; Ross, Zhang and McQuestin, 2008). As such, environmental factors that alone or in combination prevent growth of *L. monocytogenes* are of great practical interest. Table 22 lists limits for growth of *L. monocytogenes* for selected environmental hurdles relevant to RTE seafoods. The models of Meijlholm and Dalgaard (2007, 2009) can also be used to determine combinations of environmental factors that would be expected to prevent growth.

Thermal inactivation of *Listeria*: Historically, listericidal treatments have consisted principally of lethal heat treatments. The heat resistance of *L. monocytogenes* has been extensively studied in meat, milk and dairy products (ICMSF, 1996). The thermal death time curve for *L. monocytogenes* in cod and salmon was studied by Ben Embarek and Huss (1993), who reported that the heat resistance of *L. monocytogenes* is higher in salmon than in cod with $D_{60^\circ\text{C}}$ values being 4.5 min and 1.8 min, respectively. It was assumed that the higher lipid content (approximately 13 percent) of salmon protected the bacterium.

Despite some early reports, it appears that *L. monocytogenes* are not unusually heat-tolerant, and most reports of the presence of *L. monocytogenes* in foods that have received listericidal treatments are probably due to post-processing contamination. $D_{55^\circ\text{C}}$ values are in the range 1–12 min, $D_{60^\circ\text{C}}$ values are in the range 0.2–0.5 min, and $D_{65^\circ\text{C}}$ values are in the range 0.2–0.9 min. Estimates for Z-values are in the range of 4.25–5.5 °C. In drier or oilier products, or where heat penetration is impeded, D-values may be higher (Bremer, Fletcher and Osborne, 2003).

TABLE 22
Levels of environmental factors relevant to RTE seafood products required to prevent growth of *L. monocytogenes*

Environmental factor	Lower limit	Upper limit
Temperature (°C)	–2 to +4	45
Salt (% w/v water phase NaCl)	13–16	< 0.5
(& corresponding a_w)	0.92–0.93	> 0.997
pH (hydrochloric acid as acidulant)	4.2–4.3	9.4–9.5
Lactic acid (mM, water phase)		3.8–4.6 (undissociated)
Acetic acid (mM, water phase)		10.3 (undissociated)
Phenol (ppm)		32
Nitrite (ppm)		350
Carbon dioxide (ppm)		3 140

Sources: Compiled from Ross, Dalgaard and Tienungoon (2000), and Meijlholm and Dalgaard (2009).

Prevention and control: The preceding discussion has highlighted that *L. monocytogenes* is a pathogenic bacterium that is present in many environments, including those in which seafoods are processed, and that *L. monocytogenes* can cause serious illnesses, with a high fatality rate. While it is a common contaminant of RTE foods, infections from *L. monocytogenes* are rare and usually affect only people with known predisposing conditions that reduce the efficacy of their immune systems. The explanation for this apparent paradox seems to be that high doses of *L. monocytogenes* are usually required before infections are established. In other words, the main risk to consumers arises from the growth of the organism in the product rather than its mere presence. Given that *L. monocytogenes* are able to grow, albeit slowly, in many RTE foods, minimization of the presence and growth of *L. monocytogenes* in RTE foods forms the basis for risk management strategies.

Regulatory measures to protect consumers from food-borne *Listeria monocytogenes*: The demonstration that low levels of *L. monocytogenes* in foods are unlikely to cause disease has occurred in the last decade. Initially, the severity of listeriosis led many nations to establish regulations embracing the concept of “zero tolerance”, i.e. that *L. monocytogenes* must not be able to be detected in a specified number of 25 g samples of the food of interest.

Subsequently, new research and several risk assessments have indicated that low levels of *L. monocytogenes* are consumed daily by most people, apparently, with no adverse effect. In recognition of these advances in understanding of the dose vs probability of infection relationship of *Listeria*, and understanding of the potential for its growth in various RTE foods, several nations and trading blocs have moved to risk-based policies to establish criteria for *L. monocytogenes* in foods (Warriner and Namvar, 2009). Recently introduced international requirements (EC, 2005a, 2007a) and Codex guidelines (CAC, 2007b; CAC, 2009a) for control of risk from food-borne *L. monocytogenes* on RTE foods allow some tolerance for low levels of the pathogens in foods in which growth is unlikely or impossible. Specifically, these regulations and guidelines infer that foods respecting the following criteria present no significant public health risk:

- which due to their formulation and processing, prevent *L. monocytogenes* growth in the product;
- where *L. monocytogenes* levels do not exceed 100 cfu/g.

Recognizing that some products may support very limited growth of *L. monocytogenes*, an additional category is recognized, i.e. foods that during their normal shelf-life and under reasonably foreseeable conditions and duration of distribution and handling, do not support the growth of *L. monocytogenes* (CAC, 2009a) of more than 0.5 log cfu. In those foods, up to 100 cfu/g *L. monocytogenes* at the point of consumption is also considered to present no significant risk. Some variations on this principle are evident, e.g. in Australia cold-smoked salmon is required to be “free” of *Listeria monocytogenes* in five 25 g samples, but one positive sample may be tolerated provided that the level of *L. monocytogenes* in that sample does not exceed 100 cfu/g. In foods that do support its growth, most States require that *L. monocytogenes* should not be present in the product, i.e. a “zero tolerance” approach.

Industry approaches to protect consumers from food-borne *Listeria monocytogenes*: The importance of preventing growth of *L. monocytogenes* in management of the risk of listeriosis from RTE foods has created great interest in the development of product formulations, additives and packaging systems that can prevent growth of *L. monocytogenes*. There are many reports in the literature describing the efficacy of different hurdles and hurdle combinations. As noted above, the microbial ecology knowledge summarized and quantified in predictive models such as that of Meijlholm and Dalgaard (2009) provides a means of identifying promising and sensorially acceptable combinations of hurdles, or assessing existing products for

their ability to prevent *L. monocytogenes* growth without the need for expensive and time-consuming challenge studies.

Even if growth can be prevented in the product, contamination must be minimized, or contaminants eliminated, to ensure that levels do not exceed the maximum tolerable level of 100 cfu/g. Elimination of *L. monocytogenes* in RTE seafoods can only be guaranteed in products that after packaging are subjected to a listericidal process, typically a heat treatment. High-pressure processing has also been investigated, but high pressures (e.g. > 500 MPa) are required to inactivate *L. monocytogenes* and, at these pressures, physical changes can occur to the proteins of some seafoods products, leading to an unacceptable loss in quality. Similarly, heating to listericidal levels will cook the proteins in seafoods, which is unacceptable for many lightly preserved seafood products, even if postprocessing contamination can be prevented. Accordingly, factory hygiene and monitoring are important to produce foods that have acceptably low levels and frequencies of *L. monocytogenes* contamination.

Control of listeriosis can be achieved using HACCP and GHPs, with some seafood processing plants able to consistently achieve very low frequencies of contamination of final product. *L. monocytogenes* is sensitive to common cleaning and disinfecting agents, and chlorine, iodine, acid and anionic/quaternary ammonium-type sanitizers are effective against *L. monocytogenes* at concentrations of 100 ppm, 25–45 ppm, 200 ppm and 100–200 ppm, respectively. *L. monocytogenes* often hides in niches in the processing environment and great care must be taken to clean such niches. The processing plant must have a *Listeria* surveillance programme and have procedures implemented to find the source of the organism when it is detected by routine monitoring, and steps to eliminate it. Procedures to demonstrate that it has been eliminated are also required before processing recommences.

There are numerous sources of published and Internet-based information and advice on appropriate processes and procedures for *L. monocytogenes* control in RTE food processing plants.

3.2.1.7 *Staphylococcus aureus*

The genus *Staphylococcus* comprises several species, of which *S. aureus* in particular is associated with food-borne disease. The staphylococci are Gram-positive cocci bacteria. Their primary habitat is the skin, glands and mucous membranes of warm-blooded animals including humans. Approximately 30–50 percent of humans harbour *S. aureus* on their skin or mucous membranes without symptoms. Importantly, sores and scratches on the skin are often infected with *S. aureus* that can be transferred to foods by food handlers with such sores or even those who are asymptomatic carriers. *S. aureus* survives well in the environment, being relatively resistant to dryness, and it may be isolated from a range of sources that come into contact with humans and animals.

While *S. aureus* is the second-most common cause of food poisoning, it is not a major cause of seafood-borne illness. *S. aureus* is seldom isolated from fresh seafood products, but it can be found in products that are cooked and have involved extensive human handling, such as picked crab meat. When implicated in food-borne disease, it is often involved with foods that are cooked well in advance of eating, have received much manual handling, and have been subject to temperature abuse. The disease caused is usually mild and self-limiting, resolving within 24–48 h of onset of symptoms.

The disease and some epidemiological aspects: *S. aureus* produces a range of toxins and diverse disease syndromes. These include a food-borne intoxication that principally is characterized by vomiting, induced by Staphylococcal enterotoxins (SEs). Individual *S. aureus* strains produce one or more of these antigenically distinct SEs, which were originally designated from SEA to SEE. The most frequent causes of food poisoning are due to SEs A, B, C1, D, and E. However, in the last decade, more SEs have been

described and have been designated SEF, SEG, SEH, SEI, SEJ, SEK, SHL, SEM, SEN, SEO, SEP, SEQ, SEIR and SEU (Omoe *et al.*, 2005). Other species of *Staphylococcus* (*S. intermedius* and *S. hyicus*) also produce some of these enterotoxins and have, albeit rarely, been reported to have caused human food-borne illness.

Upon ingestion, the toxins cause nausea, vomiting, stomach cramps and, sometimes, diarrhoea. SEA and SEB are the best characterized, and they are also regarded as superantigens because of their direct effect on cells of that immune system, which can lead to a syndrome described as “acute toxic shock”. Staphylococcal enterotoxins, which are proteins, are preformed in the food. Thus, growth of the organisms in the food is a prerequisite for toxin production, and disease. Because the toxins are preformed in the food, the time to onset of symptoms is short, typically 1–4 h. All SEs have a molecular weight of approximately 23–27 kD, being approximately 200–250 amino acids in length. The proteins are very stable and survive normal cooking, and they are resistant to gastrointestinal proteases, such as pepsin. The toxins, if present in foods, are not sensorially detectable.

The primary effect of the toxins is a neurological (i.e. not an enterotoxic) effect that involves stimulation of afferent vagus nerves in the intestine via serotonin released by other intestinal cells due to the influence of the SEs (Hu *et al.*, 2007). While very unpleasant, the disease is self-limiting and typically lasts only 24–48 h without long-term effects. Owing to the relatively short-lived nature of the disease, it is believed that only a small fraction (1–5 percent) of cases are reported. A higher frequency is seen during warmer months, presumable a reflection of the greater likelihood and severity of temperature abuse of foods, and in November and December. The latter peak may be correlated with leftover holiday foods and buffets (Jablonski and Bohach, 1997).

Prevalence in fish and fishery products: Staphylococci may be isolated from newly caught fish, especially in warm waters (Gram and Huss, 2000). However, enterotoxigenic strains are typically transferred from food handlers with hand infections or with a cold or a sore throat. *S. aureus* has been isolated at levels of 2–10 percent in fish and bivalves but much more commonly in cooked, handled crustaceans, where as much as 24–52 percent of samples may be positive (Jablonski and Bohach, 1997). Batters used with seafoods may represent a special risk (FDA, 2011a) due to their manner of preparation and storage in food service, and because frying will not inactivate the toxin.

Growth and survival in fish and fishery products: *S. aureus* can grow at temperatures of from approximately 8 to 48 °C, pH > 4.3 and water activity (NaCl as humectant) > 0.86. Optimal levels for these conditions are ~37 °C, pH 7, a_w 0.995, respectively. In general, where growth is possible, toxin production will also be possible, although some reports indicate that SEs are not produced at temperatures < 10 °C. As with most bacteria, for any environmental condition, the range over which growth is possible is reduced when another environmental factor is suboptimal.

Although it may be detected on raw fish (and meat), *S. aureus* will not usually be able to grow to toxigenic levels. Disease-causing levels of toxin occur only when extensive growth of *S. aureus* has occurred, typically at levels $\geq 10^6$ cfu/g. Staphylococci are relatively slow-growing compared with spoilage bacteria and, under most seafood processing regimes, low temperatures and the presence of other bacterial species (spoilage organisms) prevent extensive growth of *S. aureus* from occurring. However, in low water activity products, *S. aureus* may have a competitive advantage because of its unusually high tolerance of salt. Similarly, growth and toxin production may occur in products such as cooked crustaceans where the heat-processed meat is virtually sterile and where the hand peeling operations provide ample opportunity for contamination with staphylococci. Because *S. aureus* is a mesophilic organism, some degree of temperature abuse typically also precedes intoxications.

Prevention and control: Growth and toxin formation may easily be prevented by proper chilling of products. Avoidance of cross-contamination of heat-treated (cooked) products is also important. While *S. aureus* is capable of growth anaerobically, anaerobic growth is slower and less extensive under otherwise equivalent conditions. Toxins have not been detected in canned foods.

The European Union (Member Organization) has set microbiological criteria for *S. aureus* in cooked crustaceans where none of five samples may exceed 1000 cfu/g and only two samples may exceed 100 cfu/g (EC, 2001a). Other jurisdictions are somewhat more lenient: the Canadian Food Inspection Agency (CFIA, 2011) allows up to one sample in five of any kind of seafood product to contain more than 1000 cfu/g *S. aureus* but no sample is permitted to exceed 10 000 cfu/g *S. aureus*. Similarly, the FDA will tolerate up to 10 000 cfu/g *S. aureus* in seafood products, provided that SEs are not detected (FDA, 2011b).

3.2.1.8 *Clostridium botulinum*

Clostridium botulinum is a Gram-positive, obligate-anaerobic, spore-forming, rod-shaped bacterium that is widely distributed in soils and marine sediment all over the world. It also colonizes the gastrointestinal tract of fish, birds and mammals. This organism produces a neurotoxin and, based on the antigenic characters of the neurotoxin, six types are recognized, A–F. *Clostridium argentinense* produces type G botulinum toxin. Rare strains of *C. butyricum* and *C. baratti* may produce botulinum toxins. Based on their physiology, three groups of *C. botulinum* are recognized:

- Group I includes proteolytic botulinum toxin types A, B and F that are heat resistant (D_{100} of spores ~25 min) and salt tolerant (inhibitory NaCl 10 percent) with a minimum growth temperature of 10 °C.
- Group II includes non-proteolytic botulinum toxin types B, E and F that are heat sensitive (D_{100} of spores < 0.1 min), psychrotropic (minimum growth temperature 3 °C) and salt sensitive (inhibitory NaCl 5 percent).
- Group III includes botulinum toxin types C and D that are salt sensitive (inhibitory NaCl 3 percent) with a minimum growth temperature of 15 °C.

Epidemiological aspects: Human botulinum may be caused by *C. botulinum* types A, B, E and, rarely, types F and C. Type F toxin produced by *C. baratti* and type E toxin produced by *C. butyricum* have rarely been involved in human botulinum. Strains of *C. botulinum* that produce type C and D toxins are mostly involved in botulism in non-human species. Owing to the greater heat resistance of Group I spores, cases due to this type are associated with insufficiently processed home-preserved foods such as canned vegetables and cured meat. Cases due to Group II strains that can grow at lower temperatures could be associated with mildly heated products packaged under anaerobic conditions and stored in a refrigerator, e.g. vacuum-packed smoked fish. Food-borne botulinum has been reported from several countries such as the United States of America, Canada, Japan, China, the Russian Federation, some countries in Europe, South Africa and Iran (Islamic Republic of) (Johnson, 2007). In the United States of America, 25–60 cases occur annually, mainly through home-prepared foods. In the period 1973–2006, there were 43 seafood-associated outbreaks involving 152 cases, of which 61 required hospitalization and 9 resulted in death (Iwamoto *et al.*, 2010). Eighty-six percent of the reports were from Alaska, involving consumption of traditional Alaskan native seafood dishes prepared with salmon eggs, fish heads, seal and whale meat. There were no cases associated with crustaceans or molluscs. Group II botulism associated to various foods (including fish and fishery products) in Europe and other parts of the world in the period 1980–2004 have been reviewed by Lindström, Kiviniemi and Korkeala (2006). Uneviscerated salted mullet (faseikh) was associated with an outbreak involving 91 cases in Egypt. Smoked fish were involved in 85 cases in Georgia in the period 1980–2002, and fish were involved in 72 cases

in the Russian Federation in 1999 (Lindström, Kiviniemi and Korkeala, 2006). Most fish-associated outbreaks and cases are due to type E, and type A and B botulism have generally been associated with meat products, but fish may also be involved in these, for example, cases due to type B involving sturgeon fish occurred in the United States of America in 1990 (Lindström, Kiviniemi and Korkeala, 2006).

Food-borne botulism has an incubation period of about 12–36 h following consumption of toxic food. However, this could be 2 h where foods have high levels of toxin, or as long as 2–14 days where low levels of type B or E toxins are consumed. Clinical symptoms of food-borne botulism include fatigue, weakness and vertigo, blurred vision, dilated pupils, drooping eyelids, difficulty in swallowing, weakness of neck and mouth, and paralysis of limbs and torso. Vomiting, diarrhoea and abdominal swelling may occur. In severe cases, respiratory muscles are weakened and mechanical ventilation becomes necessary to prevent death. Treatment involves mechanical ventilation to support respiration and administration of antibodies (ABE antitoxin) before neurological symptoms set in. Fatality used to be about 50 percent in the early twentieth century, but this has decreased to about 10 percent with the availability of antisera and respiratory support systems.

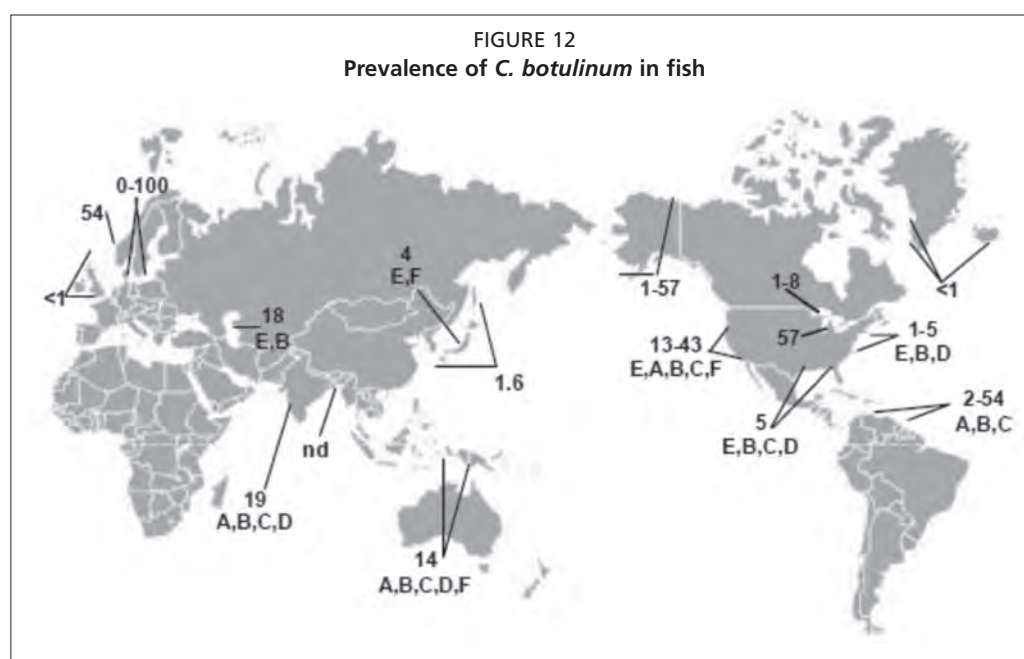
The botulinum toxin is a highly potent neurotoxin with an estimated lethal dose of 0.1–1 µg/kg by oral route. The toxin is highly stable under acidic conditions (pH 3.5–6.5), but dissociates under alkaline conditions and is inactivated. Thus, the toxin is inactivated in spoiling fish products with pH > 7.5. The toxin is heat sensitive, and inactivation of botulinum toxin in buffers and foods occurs when subjected to 70 °C for 1 h or 80 °C for 30 min or boiling for 15 min (Johnson, 2007). However, thermal inactivation does not show a log-linear effect, and considerable tailing may be observed, particularly at lower processing temperatures. This complicates the use of traditional D-values to model thermal inactivation, and it has been proposed that heat resistance be expressed as the time required for inactivation to below the threshold for toxicity (Johnson, 2007).

Ecology and occurrence in fish and fishery products: *C. botulinum* is ubiquitous and occurs in soils, sediments of aquatic environments (both freshwater and marine) and in the intestinal tracts of animals and fish in temperate, arctic and tropical environments. The organisms are saprophytic and do not have an obligatory relation with animal hosts. In the United States of America, western soils commonly have type A and eastern soils type B; in European soils, usually type B are found. In coastal regions of the world, type E can be found all over the world, and the prevalence could range from 1.2 percent to 65 percent (Johnson, 2007) and may even reach 100 percent in some areas (Figure 12). In farmed trout, levels of up to 5.3 spores/g have been found and this could be due to fish being reared in mud ponds and fed with wet feed (Huss, Pedersen and Cann, 1974). A high prevalence of spores has been reported from Poland, China, France, the United States of America and the Russian Federation, and these countries experience higher incidences of botulinum (Johnson, 2007). Although many types of meat such as beef, poultry and pork rarely contain *C. botulinum* spores, where they are part of processed food with mixed ingredients containing fish or vegetables, contamination could occur.

Generally, low numbers of spores are associated with fish (e.g. 1–2 or a few hundred spores per kilogram). Higher spore numbers, e.g. 2 000–3 000 spores/kg have been reported by some investigators (Lund and Peck, 2000). There are very limited studies on fishery products. There are some reports on prevalence in smoked fish and generally low levels (0–3 percent) have been found to be positive with numbers ranging from 40 to 290 spores/kg (Gram, 2001).

Growth and survival in fish and fishery products: Although *C. botulinum* spores are commonly found in fish, the organism is not considered a hazard in fresh fish because the levels are very low and the redox potential (Eh) of fresh fish and fishery

products is high and not favourable for germination of spores and multiplication of this organism. Aerobic bacterial flora associated with fish would cause spoilage before growth and toxin production by *C. botulinum* can occur. As botulism is an intoxication, the hazard is with foods that permit spore germination, spore growth and toxin production by the organism. The risk of toxin formation is considered high in smoked fish, as the heating step is not adequate to eliminate the spores. Cold-smoked fish have low salt levels and are often vacuum-packed, and these conditions are favourable for the growth of *C. botulinum*.



Sources: From Huss (1980) and Lalitha and Surendran (2002).

The main factors that control the growth of *C. botulinum* in foods are temperature, pH, salt, water activity (a_w), redox potential and the presence of any preservatives. The organism is strictly anaerobic and sensitive to oxygen. *C. botulinum* type E can grow and produce toxin at 3.0–3.3 °C or in up to 5 percent NaCl (water phase salt) when other growth conditions are optimal (Gram, 2001). Tolerance to water activity could vary depending on the solute, e.g. 0.97 with NaCl, and 0.94 with glycerol for strains of Group II. For stains of Group I, the minimum a_w is 0.9353. The organism is sensitive to acid, and strains of Group II do not grow at pH below 5.0, while strains of Group I do not grow below pH 4.6. The growth-limiting factors are indicated in Table 23.

TABLE 23
Growth-limiting factors for *C. botulinum*

Parameters	<i>C. botulinum</i> Group I	<i>C. botulinum</i> Group II
Growth temperature		
Minimum	10 °C	3 °C
Optimum	35–40 °C	18–25 °C
Maximum	48 °C	45 °C
Minimum pH	4.6	5.0
Inhibitory a_w	0.9353	0.97
Inhibitory NaCl	10%	5%
D _{100°C} of spores	~25 min	<0.1 min
D _{121°C} of spores	0.21 min	<0.005 min

It needs to be emphasized that the factors indicated in Table 23 seldom function independently. For example, toxin production could be inhibited at salt concentrations of less than 5 percent where the temperature is less than optimum. The minimum a_w could vary with solute, e.g. 0.97 when NaCl is used and 0.94 when glycerol is used.

The toxins produced by *C. botulinum* are thermolabile, although it is not considered acceptable to rely on cooking to eliminate the hazard in foods (Gram, 2001). Thermal inactivation depends on other factors. For example, *C. botulinum* type E toxin is more heat resistant at lower pH values (pH 4.0–5.0). The toxin was inactivated after 5 min at 60 °C in a cooked meat medium of pH 7.5, but at 65 °C in meat broth of pH 6.2. In canned corn of pH 6.2, the 3D reduction occurred in 2 min at 74 °C, but in a phosphate buffer of 6.8, a similar reduction occurred in 1 min, which extended to 6 min in the presence of 1 percent gelatin.

A combination of factors is used to control *C. botulinum* in foods. The inhibitory factors used are thermal treatment, pH, water activity, salts or other inhibitors and competitive flora. Low-acid foods with $\text{pH} \leq 4.6$ have an excellent safety record. However, certain conditions in low-acid foods may permit growth of *C. botulinum*, e.g. inadequate penetration of acids leading to formation of microenvironments with higher pH or metabiosis – where fungal mats form on surface of foods increasing pH under the mats (Johnson, 2007). Control of water activity through addition of salt is another method used to control *C. botulinum*. Temperature (< 10 °C for Group I and < 3 °C for Group II) is also used in combination with another inhibitory factor such as salt. Fish is considered an excellent substrate for the growth of *C. botulinum*. Cann and Taylor (1979) studied toxin production in farm-produced, hot-smoked whole trout with about 80 percent prevalence for *C. botulinum*. No toxin production was detected in fish with 2.5 percent salt when stored at 10 °C for 30 days, but at 2.0 percent salt, whole ungutted fish became toxic.

Freezing does not affect spore viability, but under frozen conditions, germination and growth of *C. botulinum* does not occur and, if vacuum packed, frozen storage will have little effect on lipid oxidation and, consequently, sensory quality. At low-temperature storage, other factors such as salt (5 percent water phase salt) are required to prevent germination and growth of *C. botulinum* type E. Toxin production is prevented for 2–3 weeks at 2–2.5 percent salt and for 4–5 weeks at 3–3.5 percent salt at 5 °C in laboratory media inoculated with high inocula (10^4 – 10^5 spores/ml). On the other hand, with fish as substrate, toxin production was slower. In cold-smoked trout with 1.7 percent salt, no toxin was detected when stored at 4 °C or 8 °C for 4 weeks (Dufrense *et al.*, 2000), and in hot-smoked trout stored at 10 °C, no toxin was detected at 2.5–3.5 percent salt (Cann and Taylor, 1979). Thus, 3.5 percent salt combined with a maximum storage of 4 weeks at 4 °C is considered safe for vacuum-packed cold-smoked fish (Gram, 2001).

Although *C. botulinum* is sensitive to oxygen, toxin production could occur in air-packed fish at 5–6 days compared with 4–5 days in vacuum-packed fish. Microenvironments could exist in air-packed fish, where the organism could grow and produce toxin. However, shelf-life is reduced by a factor of 1.5–2.0 by aerobic storage compared with vacuum-packed storage. In some countries, vacuum-packed cold-smoked fish should contain 3.5 percent NaCl or 3.0 percent combined with 200 ppm nitrite, but only 2.5 percent salt is required for aerobically packed fish (Gram, 2001). Although spoilage is relied upon as a safeguard in aerobic-packed fish, it is now accepted that oxygen is no safeguard against *C. botulinum* toxin formation. Variable results have been obtained with 1 percent sorbate.

Thermal inactivation of *C. botulinum* spores has been studied extensively, particularly from the point of view of the canning industry. D-values for the two groups of *C. botulinum* vary (Table 23). For non-proteolytic types, heat treatment of 90 °C for 10 min has been reported to provide a safety factor of 10^6 (6-D process).

For the proteolytic group, the canning industry generally uses a D-value of 0.2 min at 121 °C for calculating the thermal process, and for most heat-resistant strains, z-values (temperature change necessary to bring about a tenfold change in the D-value) are taken as 10 °C (Martens, 1999; Austin and Dodds, 2001).

Prevention and control: In the canning industry, a 12D process (12 log reduction in spore count) is used as a minimum heat process to be applied to low-acid canned foods. For proteolytic strains, this would be $12 \times 0.2 = 2.4$ min at 121 °C (also called F-value). Considering spores with higher resistance ($D_{121^\circ\text{C}} 0.25$), this would be $12 \times 0.25 = 3.0$ and, in commercial practice, higher F-values (e.g. 5.0) are often used to produce botulism-safe canned food. For foods that do not receive a thermal process compared with canning, a combination of temperature and salt (as discussed above) is used.

3.2.1.9 *Escherichia coli*

Escherichia coli is a member of the family Enterobacteriaceae and is a common inhabitant of the intestinal tract of humans and warm-blooded animals. This species is serologically very diverse with more than 173 somatic (O) antigens, 56 flagellar (H) antigens and 103 capsular (K) antigens (Meng *et al.*, 2007). Although most *E. coli* strains are commensals in the intestinal tract, some strains that have acquired certain virulence genes can cause gastrointestinal illness. Based on clinical syndromes and virulence properties, diarrheagenic *E. coli* have been categorized as: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffuse-adhering *E. coli* (DAEC). Major serovars associated with different pathotypes are indicated in Table 24.

TABLE 24
Major serogroups associated with different pathotypes of *E. coli*

Pathotype	Major serogroups
EPEC	O55, O86, O111ab, O119, O125ac, O126, O127, O128ab, O142
ETEC	O6, O8, O15, O20, O25, O27, O63, O78, O85, O115, O128ac, O148, O159, O167
EHEC	O157:H7, O26, O111, O103, O121, O45, O145
EIEC	O28ac, O29, O112, O124, O136, O143, O144, O152, O164, O167
EAEC	O3, O15, O44, O77, O86, O92, O111, O127
DAEC	O1, O2, O21, O75

Source: Meng *et al.* (2007).

Epidemiological aspects: ETEC infections are associated with children's diarrhoea in developing countries and with traveller's diarrhoea in industrialized countries. Contaminated food and water have been implicated in the cases, and the infectious dose seems to be fairly high with 108 cfu causing high attack rates in volunteers (Nataro and Kaper, 1998). EPEC is associated with diarrhoea in infants, and the reservoir of infection has been reported to be symptomatic or asymptomatic children and asymptomatic adults. Contaminated weaning food is a common vehicle of infection. EHEC infections may manifest as non-bloody diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome (HUS). Serovar O157:H7 was the first to be recognized as EHEC, but subsequently other serotypes (Table 24) have also been classified as EHEC. Outbreaks have been associated with undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized fruit juice, sprouts and vegetables (Feng, Weagant and Jinneman, 2011). A large outbreak with more than 3 800 cases and 54 fatalities associated with sprouts occurred in Germany in 2011 involving *E. coli* O104:H4, a serovar that was earlier classified as an enteroaggregative type. It is suspected that this serovar acquired genes of EHEC through lateral gene transfer (Werber *et al.*, 2012). There has been only one outbreak suspected to be linked to salmon roe in Japan, and identical genotypes of *E. coli* O157: H7 were isolated from clinical cases and salmon

roe (Asai *et al.*, 1999). This serovar has a low infectious dose (< 100 cells), and infected persons could shed the bacteria in faeces up to 13–21 days after the onset of symptoms; but in rare cases, this could extend to two months (Meng *et al.*, 2007).

DAEC have been associated with children of 1–5 years of age while EAEC have been associated with diarrhoea in both children and infants. DAEC infections are characterized by mild diarrhoea without blood or leucocytes. EIEC cause Shigella-like dysentery or non-bloody diarrhoea. Because of their involvement in several food-borne outbreaks, EHEC have been the most studied of the diarrhoeagenic *E. coli*.

Association with fish and fishery products: As the primary reservoirs of diarrhoeagenic *E. coli* are humans or cattle, these bacteria could reach fish and fishery products that are contaminated with sewage, farm wastes or non-potable water. ETEC has been isolated from seafood in Brazil (Teophilo *et al.*, 2002). Detection of shiga toxinogenic *E. coli* (STEC) and even O157:H7 serovar in seafoods in India have been reported (Kumar *et al.*, 2004; Surendraraj, Thanpuran and Joseph, 2010). Surveillance programmes in the European Union (Member Organization) in 2010 indicated the presence of STEC in 4.2 percent of fishery products in Spain (EFSA/ECDPC, 2012).

Growth and survival in seafoods: Although *E. coli* is a mesophilic organism with an optimum temperature for growth of 35–40 °C, some pathogenic strains grow at temperatures as low as 7 °C and as high as 46 °C. The EHEC strains have a minimum growth temperature of 8 °C and maximum of about 44–45 °C (ICMSF, 1996). Diarrhoeagenic *E. coli* have been reported to survive well at refrigeration temperatures (3–7 °C) with reductions over 1–5 weeks ranging from 100.5 to 101.5 (ICMSF, 1996). *E. coli* strains are known to be sensitive for salting (can grow in 6 percent sodium chloride) and drying, but EHEC strains may show acid tolerance depending on the type of acid present, e.g. showing growth in medium adjusted to pH 4.5 with hydrochloric acid, but with lactic acid (ICMSF, 1996). Diarrhoeagenic *E. coli* are readily inactivated by heating with D-values ranging from 0.75 to 0.79 min in ground beef and milk (ICMSF, 1996).

3.2.1.10 *Aeromonas and Plesiomonas*

Aeromonads are Gram-negative facultatively anaerobic bacteria that are widely distributed in the environment and in association with invertebrates, vertebrate animals and humans. The taxonomy of this group has been complex and is undergoing rapid changes. The second edition of *Bergey's Manual of Systematic Bacteriology* includes the genera *Aeromonas*, *Oceanimonas* and *Tolumonas* in the family Aeromonadaceae (Martin-Carnahan and Joseph, 2005). Traditionally, *Aeromonas* consisted of two species, mesophilic *A. hydrophila* and psychrophilic *A. salmonicida*. Subsequently, three mesophilic species were recognized, *A. hydrophila*, *A. sobria* and *A. caviae*, each consisting of several hybridization groups that can be differentiated based on deoxyribonucleic acid (DNA)-DNA hybridization test. Currently, there are 24 validly published species under the genus *Aeromonas*, of which 11 are considered clinically significant (Janda and Abbot, 2010) (Table 25). However, taxonomists have been questioning some of the species, based on DNA reassociation kinetic information or a lack of information on ecology.

Plesiomonas can be differentiated from *Aeromonas* by phenotypic characters such as the L-histidine decarboxylase test (Galindo and Chopra, 2007). Based on 76 O- and 41 K- antigens, more than 100 serovars of *P. shigelloides* have been described. Several serovars react with *Shigella* antisera. This organism is commonly isolated from the aquatic environment.

TABLE 25

***Aeromonas* spp. of clinical significance and their association with aquatic environment and food**

<i>Aeromonas</i> spp.	Freshwater	Saline waters	Foods
<i>A. hydrophila</i>	+++	++	++
<i>A. salmonicida</i>	++	0	0
<i>A. media</i>	+	0	0
<i>A. caviae</i>	++	+++	+++
<i>A. veronii</i>	±	++	++
<i>A. schubertii</i>	0	0	±
<i>A. jandaei</i>	±	0	0
<i>A. trota</i>	0	0	±
<i>A. bestiarum</i>	++	0	0
<i>A. popoffii</i>	+	0	0
<i>A. tecta</i>	0	0	0

0 = not reported; ± = rare; + = uncommon; ++ = common; +++ = predominant.

Source: Based on Janda and Abbot (2010).

Epidemiological aspects: Although *Aeromonas* spp. have been isolated from diarrhoeic stools, their role in causing gastrointestinal disease is still debated. The organism can be isolated from the faeces of asymptomatic persons with carriage rates varying from 3 percent in the tropics to 30 percent in tropical and developing countries (Galindo and Chopra, 2007). Several large-scale retrospective or prospective investigations on bacterial diarrhoeas indicate that aeromonads are associated with stools of 0.5–16.9 percent of ill persons and 0–10 percent of controls (Janda and Abbot, 2010). From 75 to 89 percent of gastroenteritis cases where *Aeromonas* has been reported to be the sole pathogen isolated are characterized by abdominal pain, mild diarrhoea and low-grade fever, while 3–22 percent of cases present dysenteric forms with symptoms including abdominal cramps, blood and mucus in stool (Janda and Abbot, 2010). Some studies suggest that persons with haematological cancers, tumours or other pathological anomalies of the gastrointestinal tract are predisposed to infections. On extremely rare occasions, *Aeromonas* has been associated with cholera-like disease (Janda and Abbot, 2010). Aeromonads may also be associated with extra-intestinal infections.

P. shigelloides has been associated with traveller's diarrhoea, which may last for 1–7 days and is often accompanied by abdominal cramps, vomiting and some degree of dehydration. Association of this organism with diarrhoea varies in different regions, e.g. 1.3 percent in Latin America and the Caribbean, 2.5 percent in Africa and 4.8 percent in South Asia (Shah, Dupont and Ramsey, 2009). This organism may also be associated with extra-intestinal infections. For gastrointestinal illness, the incubation period has been reported to be 20–24 h. Up to 12.6 percent of *P. shigelloides* infections have been reported to be associated with seafood consumption. Consumption of raw oysters and shrimps has been associated with the risk of infection (Butt, Aldridge and Sanders, 2004).

Association with fish and fishery products: Aeromonads are ubiquitous and are detected in more than 90 percent of aquatic habitats sampled in some regions (Janda and Abbot, 2010). They have been isolated from rivers, lakes, estuaries, drinking-water, groundwater and sewage. They can also be recovered from the epipelagic layer (< 200 m) of oceans, but they are more common in estuaries, where they are associated with various shellfish, and levels ranging from 102 to 106 cfu/100 ml have been reported. They are part of the natural flora of fish from warm waters.

P. shigelloides is also associated with both freshwater and marine environments and, hence, can be isolated from various fish. Although the organism is mesophilic, it has been isolated from waters in temperate environments.

Growth and survival in seafoods: *Aeromonas* is psychrotrophic with a 1–3 log increase in numbers observed in fish stored at 5 °C for 1 week. The minimum pH for growth is < 4.5, and the maximum sodium chloride concentration is 5–6 percent. *Aeromonas* is sensitive to elevated temperature with a $D_{51^{\circ}\text{C}}$ of 2.3 min (ICMSF, 1996). For *P. shigelloides*, the minimum growth temperature is 8 °C and the pH range is 4–9. Maximum sodium chloride for growth is 5. The organism is sensitive to heat, with pasteurization at 60 °C for 30 min being effective in inactivating it.

3.2.2 Histamine and other biogenic amines (Lahsen Ababouch, Jette Emborg and Paw Dalgaard)

In small physiological doses, histamine is a necessary and desirable substance involved in the regulation of critical functions in the human body, e.g. the release of stomach acid. However, large amounts of histamine and other biogenic amines in food can be toxic.

In fish products, histamine and other biogenic amines are produced by enzymatic decarboxylation of the corresponding free amino acid (Table 26). The decarboxylases are produced by specific bacteria.

TABLE 26

Amino acid precursors and biogenic amines formed in food products

Amino acid precursor	Biogenic amine
Histidine	Histamine
Ornithine	Putrescine
Putrescine ¹	Spermidine
Lysine	Cadaverine
Tyrosine	Tyramine
Arginine	Agmatine

¹ Not an amino acid.

In order to cause histamine fish poisoning (HFP), it is necessary that:

- the fish muscle contains free histidine as substrate for histamine formation;
- the fish contains and/or becomes contaminated with bacteria capable of decarboxylating histidine and possibly other amino acids;
- the product characteristics and storage conditions allow growth of histamine-producing bacteria (HPB) to high concentrations of about 10 million cells per gram or more;
- consumers actually eat fish that contain high concentrations of histamine and possibly also other biogenic amines.

Consequently, control of HFP can be achieved by eliminating one or more of these steps.

With respect to toxicity of fish products, histamine is more important than other biogenic amines. High concentrations of biogenic amines other than histamine can cause disease and discomfort, but for healthy people the concentrations of biogenic amines found in fish products are usually not toxic (Taylor, 1990; Glória, 2006). However, for sensitive individuals, a very small dose of tyramine can cause migraine headaches. For these persons, an intake of no more than 5 mg tyramine per meal has been recommended (Caston *et al.*, 2002; McCabe, 1986; Walker *et al.*, 1996). Typically, fish products contain less than 5 mg of tyramine per kilogram and therefore represent no problem even for sensitive individuals. However, products involved in some incidents of HFP have contained 150 mg of tyramine per kilogram; thus, the content of a typical 100 g fish portion can be critical. Much higher concentrations of tyramine can be found in certain cheeses, sausages and yeast extract. Chocolate can cause migraine for individuals susceptible to phenylethylamine (Glória, 2006).

3.2.2.1 Histamine fish poisoning – disease, epidemiology and implicated products

Histamine fish poisoning is an intoxication that can be caused by consumption of many different types of marine finfish, but neither freshwater fish, crustaceans or molluscan shellfish seems to cause this disease. Histamine fish poisoning is common and occurs worldwide (Table 27).

TABLE 27

Incidents and cases of histamine fish poisoning in various countries

Country	Year	Incidents or outbreaks	Cases	Annual no. of cases per million people ¹
Hawaii, United States of America	1990–2003	111	526	31.0
Denmark	1986–2005	64	489	4.9
New Zealand	2001–2005	11	62	3.1
Japan	1970–1980	42	4 122	3.2
	1994–2005	68	1 523	1.1
France	1987–2005	123	2 635	2.5
Finland	1983–2005	41	162	1.3–2.1
Taiwan Province of China	1986–2001	8	535	1.5
Norway, United Kingdom, South Africa and Switzerland	1966–2004	608	1 460	0.4–0.8
Australia, Canada, Netherlands, Philippines, Sweden and United States of America (states other than Hawaii)	1973–2005	603	3 214	0.2–0.4

¹ To compare data between regions of different population size and for different recording periods, the annual number of cases per million people was calculated.

Source: Dalgaard and Emborg (2009).

The incubation time for HFP is short (from a few minutes up to 2 h) and people often develop symptoms while they are still eating. This facilitates attribution of disease to the fish consumed, but the occurrence of HFP is under-reported because:

- many countries do not collect data on incidents of HFP;
- symptoms can be mild and of short duration, so a physician may not be contacted;
- HFP symptoms can be incorrectly identified and recorded, e.g. as a food allergy;
- some statistics exclusively include cases that are reported as a part of an outbreak (where two or more people become ill), but for HFP single cases are common.

The primary symptoms of HFP are cutaneous (rash, urticaria, oedema, and localized inflammation), gastrointestinal (nausea, vomiting and diarrhoea), haemodynamic (hypotension) and neurological (headache, tingling, oral burning and blistering sensation, flushing and perspiration, and itching). More serious complications such as cardiac palpitations occur but are rare (Taylor, 1986; Lehane and Olley, 2000). Symptoms can be resolved by antihistaminic drugs (antihistamines). These drugs block the binding of histamine to specific receptors and thereby its effect and HFP symptoms (Parsons and Ganellin, 2006; Glória, 2006).

Shalaby (1996) reviewed the oral toxicity to humans of histamine and other biogenic amines. Based on this analysis, the following guideline levels for histamine content of fish were suggested:

- < 50 mg/kg safe for consumption
- 50–200 mg/kg possibly toxic
- 200–1 000 mg/kg probably toxic
- > 1 000 mg/kg toxic and unsafe for human consumption

More recently, an extensive study found 90 percent of 1998 HFP cases were due to fish products with more than 500 mg of histamine per kilogram (Table 28). Data from fish products implicated in 30 different HFP incidents also showed that the concentration of histamine was ten times higher than the sum of the concentrations of other biogenic amines (Dalgaard *et al.*, 2008). With a meal size of 100 g, these data suggest that HFP is caused by an intake of more than 50 mg of histamine (100–500 mg being most common) together with more than 5 mg of other biogenic amines (10–50 mg being typical).

A recent Joint FAO/WHO Expert Meeting on Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products identified 50 mg of histamine as the “no observable adverse effect level” (NOAEL) derived from outbreak studies. The benchmark dose assessment methodology also identified 50 mg of histamine per meal as the dose where adverse effects are not observed. Using available fish and fishery product consumption data combined with expert opinion, the meeting agreed that a serving size of 250 g captured the maximum amount eaten in most countries at a single eating event. Based on the hazard level of 50 mg of histamine and serving size of 250 g, the maximum concentration of histamine in that serving was calculated to be 200 mg/kg.²

TABLE 28

Overview of outbreaks (n = 142) and cases (n = 1 998) of histamine fish poisoning as a function of the concentration of histamine in different seafoods

Histamine (mg/kg)	Outbreaks		Cases		Seafood or fish species
	Number	%	Number	%	
> 5 000	14	10	98	5	Escolar, kahawai, kingfish, marlin, saury, tuna, yellowfin tuna
1 000–5 000	66	47	937	47	Amberjack, anchovy, bluefish, cape yellowtail, castor oil fish/escolar, kahawai, mackerel, mahi-mahi, marlin, pilchard, red tuna, sailfish, sardine, swordfish, tuna
500–1 000	26	18	772	39	Anchovy, garfish, kahawai, mahi-mahi, mackerel, marlin, sardine, tuna
< 500	36	25	191	10	Anchovy, bonito, escolar, mackerel, mahi-mahi, pilchard, red tuna, sardine, skipjack, salmon, tuna

Source: Dalgaard *et al.* (2008).

Information from an HFP outbreak caused by escolar showed that persons consuming less than 113–215 mg of histamine experienced fever symptoms that were of shorter duration than persons consuming more of the fish and thereby higher amounts of histamine (Feldman *et al.*, 2005). In some challenge studies with human volunteers, 67.5–300 mg of histamine administered in water, grapefruit juice or fish resulted in no or mild symptoms only. However, it has also been found that 180 mg of histamine resulted in severe headache and flushing (Motil and Scrimshaw, 1979; Van Gelderen *et al.*, 1992). Thus, available data from challenge studies with human volunteers suggest pure histamine cannot always explain the toxicity of histamine-containing seafood. This apparently low toxicity of pure histamine may, to some extent, be explained by variability in the sensitivity among the few volunteers used in these studies and the relatively low amounts of histamine (< 100–500 mg) sometimes evaluated. In addition, two different hypotheses to explain the apparently low toxicity of histamine have been extensively discussed in the scientific literature.

² www.fao.org/fileadmin/user_upload/agns/news_events/1_FAO-WHO_Expert_Meeting_Histamine.pdf

The histamine-potentiator hypothesis is based on numerous experiments with laboratory animals where various compounds (agmatine, cadaverine, β -phenylethylamine, putrescine, trimethylamine, tyramine, combinations of these compounds and ethanol) inhibited normal histamine-metabolizing enzymes (histamine-N-methyltransferase, monoamine oxidase and diamine oxidase [or histaminase]) and thereby increased the oral toxicity of histamine (Taylor and Lieber, 1979; Hui and Taylor, 1985; Lyons *et al.*, 1983; Satter and Lorenz, 1990). However, data for humans are limited and do not clearly confirm the histamine-potentiator hypothesis (Taylor, 1986; Lehane and Olley, 2000; Van Gelderen *et al.*, 1992). Van Gelderen *et al.* (1992), for example, found that 22 mg of cadaverine and 18 mg of putrescine were unable to potentiate the oral toxicity 88–90 mg of histamine when tested on eight volunteers.

The mast-cell-degranulation hypothesis suggests seafood that causes HFP should contain compounds that trigger a release of histamine from mast cells in the human intestinal tissue. The HFP symptoms would then be due to indigenous histamine rather than to histamine in seafood (Taylor, 1986; Ijomah *et al.*, 1991; Clifford *et al.*, 1991; Lehane and Olley, 2000; Arnold and Brown, 1978). Evidence to support this hypothesis is very limited. In fact, compounds including tryptase and prostaglandin D₂ are released from mast cells when degranulated. However, these compounds have not been detected in serum or urine from patients with HFP (Morrow *et al.*, 1991; Sanchez-Guerrero, Vidal and Escudero, 1997).

Many species of marine finfish have caused HFP (Table 28) and the intoxication is often referred to as scombroid or scombrototoxin poisoning because of the frequent association of the illness with the consumption of scombroid fish such as tuna (*Thunnus* spp.), skipjack (*Katsuwonus pelamis*), saury (*Kololabis saira*) and mackerel (*Scomber* spp.). However, non-scombroid fish such as anchovies (*Engraulis* spp.), bluefish (*Pomatomus* spp.) escolar (*Lepidocybium flavobrunneum*), garfish (*Belone belone*), herring (*Clupea* spp.), kahawai (*Arripis trutta*), mahi-mahi (*Coryphaena* spp.), marlin (*Makaira* spp.), pilchards (*Sardina pilchardus*), sardines (*Sardinella* spp.) and swordfish (*Xiphiidae*) have also been implicated in outbreaks of this illness.

Considering that information on fish species that could be involved in HFP should be easily accessible to support risk management, the recently held Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products developed the most comprehensive list of fish available to date, and this list can be accessed on the FAO website³.

These fish species have significant amounts of histidine in their muscle tissue, where it serves as a substrate for bacterial histidine decarboxylase and formation of histamine. It seems that HFP is caused primarily by histamine rather than by other biogenic amines. Consequently, to reduce HFP, efforts to reduce growth and activity of HPB should be the main objective.

3.2.2.2 Histamine-producing bacteria

The kinetics of histamine formation during storage of seafood are sometimes characterized by a long phase with little or no histamine production, followed by a second phase where the concentration can increase rapidly (an example is shown in Figure 13). The first phase corresponds to the time needed for the specific HPB to reach high concentrations, and the length of this phase depends primarily on the initial concentration of these bacteria, their growth rate and temperature. The rate of histamine formation during the second phase corresponds to the activity of high concentrations of the HPB and it is influenced by storage conditions and product characteristics (Figure 13). Information about the bacteria that produce histamine in seafood is important. First, to reduce histamine formation, it is essential to

³ Ibid.

inhibit growth of the specific bacteria that actually produce this compound. Second, microbiological methods for seafood inspection must target the bacteria of importance and, therefore, the characteristics of these bacteria need to be known.

The bacteria responsible for histamine formation in seafood that actually caused HFP have been identified, but only in a very limited number of studies (Table 29). Prior to 2004, many were of the opinion that HFP was caused exclusively by the activity of mesophilic HPB in temperature-abused products (Kim *et al.*, 2004). However, toxic concentrations of histamine are frequently formed in naturally contaminated fish products when these are stored in ice and at chill temperatures between -1°C and $+5^{\circ}\text{C}$. A comprehensive study of 124 storage trials with naturally contaminated seafood at various temperatures found toxic concentrations of histamine (above 500 mg/kg) in 26 of 59 products stored at between -1°C and $+5^{\circ}\text{C}$ (Ababouch *et al.*, 1991; Emborg, 2007; Dalgaard *et al.*, 2008).

The importance of psychrotolerant HPB has now been recognized, and a new psychrotolerant, strongly histamine-producing species within the genus *Morganella* has been identified (Emborg, Dalgaard and Ahrens, 2006). Today, both mesophilic bacteria (*Morganella morganii*, *Hafnia alvei* and *Raoultella planticola*) and the psychrotolerant bacteria (*Morganella psychrotolerans* and *Photobacterium phosphoreum*) have been identified as responsible for histamine formation in seafood that actually caused HFP (Table 29). Several other species of bacteria are most likely to be important for histamine formation in fish products but these have not yet been related to illness, owing to the very limited number of HFP incidents where the bacteria responsible for histamine formation have been studied (Table 29).

TABLE 29

Incidents of histamine fish poisoning where the bacteria responsible for histamine formation have been identified

Implicated seafood	Bacterium	Year reported and region
Mesophilic bacteria		
Fresh tuna	<i>Morganella morganii</i>	1956 Japan
Fresh tuna	<i>Hafnia alvei</i>	1967 Czechoslovakia
Fresh tuna	<i>Morganella morganii</i>	1973 Japan
Fresh tuna	<i>Raoultella planticola</i>	1978 United States of America
Tuna heated in flexible film	<i>Morganella morganii</i>	2006 Denmark
Psychrotolerant bacteria		
Dried sardines	<i>Photobacterium phosphoreum</i>	2004 Japan
Tuna in chilli-sauce	<i>Morganella psychrotolerans</i> and/or <i>Photobacterium phosphoreum</i>	2005 Denmark
Cold-smoked tuna	<i>Photobacterium phosphoreum</i>	2006 Denmark
Cold-smoked tuna	<i>Morganella psychrotolerans</i>	2006 Denmark
Fresh tuna	<i>Photobacterium phosphoreum</i>	2006 Denmark

Source: Dalgaard *et al.* (2008).

In living bacteria, histidine decarboxylase (HDC) functions in cooperation with a membrane exchanger that allows histidine to be transported into the cell and histamine to be transported out of the cell (Molenaar *et al.*, 1993). The function of histamine formation in bacterial metabolism is not clear. Excretion of histamine may generate metabolic energy or be involved in an acid stress response (Lucas *et al.*, 2005; Van Poelje and Snell, 1990; Molenaar *et al.*, 1993).

Histidine is the only amino acid so far known for which decarboxylases of two different types have evolved (Van Poelje and Snell, 1990; Tanase, Guirard and Snell, 1985). One type is the pyridoxal 5'-phosphate-dependent HDC. This HDC has been isolated and characterized from Gram-negative bacteria (*M. morganii* [Tanase, Guirard and Snell, 1985], *Raoultella planticola* [Guirard and Snell, 1987; Kanki *et al.*, 2007],

Enterobacter aerogenes [Guirard and Snell, 1987], *P. phosphoreum* [Morii and Kasama, 2004; Morii and Kasama, 1995; Kanki *et al.*, 2007] and *Photobacterium damsela* JCM 8968 [Kanki *et al.*, 2007]). The other type of HDC is the pyruvoyl-dependent HDC produced by Gram-positive bacteria. This enzyme has been isolated from the following bacteria: *Lactobacillus* 30a (Hackert *et al.*, 1981), *Lactobacillus hilgardii* 0006 (Lucas *et al.*, 2005), *Leuconostoc oeni* IOEB (Coton *et al.*, 1998), *Tetragenococcus muriatricus* (Konagaya *et al.*, 2002) and *Clostridium perfringens* (Huynh and Snell, 1985). No organism able to produce HDC of both types is yet known (Van Poelje and Snell, 1990).

Strains of some Gram-positive bacteria that can produce histamine have been isolated from salted, dried or fermented foods (Landete, Pardo and Ferrer, 2006). However, Gram-positive HPB have not yet been identified as responsible for histamine formation in fish products that actually caused HFP (Table 29). A wide range of Gram-negative bacteria isolated from seafood are able to produce histamine. However, only a minor part are able to produce histamine in high concentrations (> 1 000 mg/kg) even under optimal conditions. These bacteria have been designated prolific histamine producers (Behling and Taylor, 1982).

Some HPB such as *P. phosphoreum* are part of the natural microflora in seawater, and a part of the natural flora in the intestines, gills and on the skin of fresh fish (see review by Dalgaard, 2006). They invade fish flesh from these reservoirs. This is reflected by higher histamine concentrations in fish flesh adjacent to gills and intestines and higher histamine concentrations in undressed as compared with dressed fish (Frank, Yoshinaga and Nip, 1981; Kim, An and Price, 1999; Kim *et al.*, 2001; López-Sabater *et al.*, 1996; Taylor and Speckhard, 1983). Enterobacteriaceae in fish products often result from post-harvest contamination as these HPB are found in water, baskets and floors/equipment at fish processing plants and fish markets (Corlett, Jeffrey and Niven, 1978; Subburaj, Karunasagar and Karunasagar, 1984).

Niven's agar (Niven, Jeffrey and Corlett, 1981) has been used for enumeration of HPB. However, this method relies on pour plating (with 45 °C warm agar) and incubation of plates at 37 °C. Consequently, Niven's agar will detect neither *P. phosphoreum* nor *M. psychrotolerans* as these bacteria do not grow at 37 °C. In addition, Niven's agar has been associated with false positive results. Therefore, results must be interpreted carefully (Lehane and Olley, 2000).

Various PCR methods to detect the gene encoding for histidine decarboxylase (*hdc*) have been developed (Landete *et al.*, 2007). Primer sets for the detection of *hdc* in both Gram-negative and Gram-positive bacteria are available. In addition, a PCR assay to detect the four most important decarboxylase genes (histidine, tyramine, putrescine and cadaverine) from a wide range of Gram-positive and Gram-negative bacteria associated to food has been suggested (De las Rivas *et al.*, 2006). The ability of PCR methods to differentiate between weakly and strongly HPB deserves further study. This is important if PCR methods are to be used in seafood inspection as detection of weak HPB might lead to unnecessary concerns.

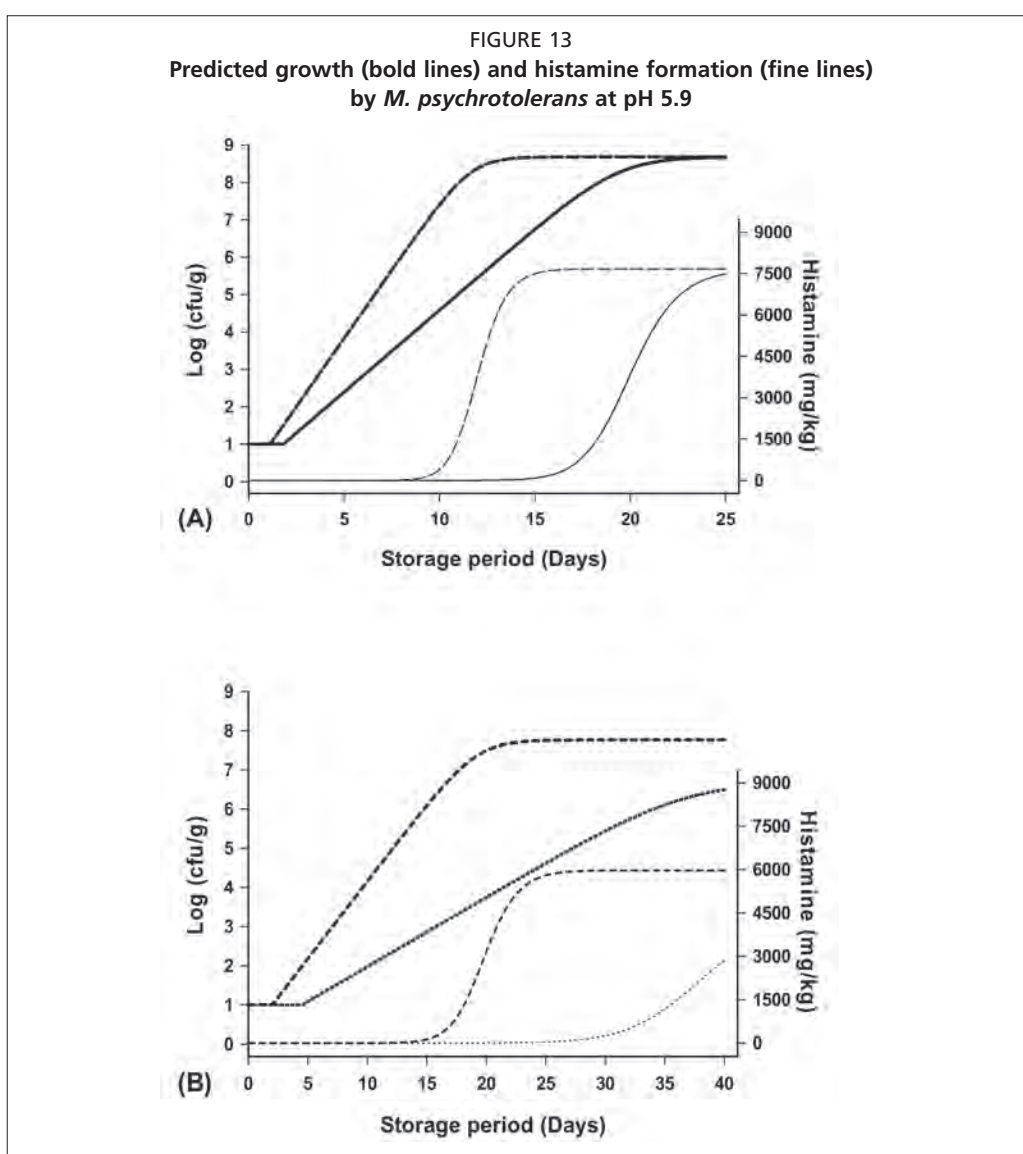
3.2.2.3 Prevention and control

Despite decades of research, efforts by the seafood sector and efforts by national and international authorities, HFP remains common. This indicates available information is either incomplete or not used appropriately to manage this seafood safety issue (Dalgaard *et al.*, 2008).

Growth of HPB, and the related formation of histamine, depends on several factors including their presence in a specific seafood, storage conditions (temperature, atmosphere) and various product characteristics (pH, lactic acid, salt, smoke components and added antimicrobial agents) (Figure 13). To control HFP, it is important to know the effect of these parameters on the most important HPB. The information can be

used, for example, to determine safe the shelf-life or to obtain a desired shelf-life by changing storage conditions or product characteristics.

Chilling of fish and fish products is highly important to increase the time to formation of critical histamine concentrations. Below 7–10 °C, mesophilic and strongly HPB do not form toxic concentrations of histamine in fish products. However, the psychrotolerant bacteria *M. psychrotolerans* and *P. phosphoreum* can produce toxic concentrations of histamine at 0–5 °C (Dalgaard *et al.*, 2006; Emborg, Laursen and Dalgaard, 2005; Kanki *et al.*, 2004; Okuzumi, Okuda and Awano, 1982). Simple empirical models to predict histamine formation have been suggested (Frank, 1985; Frank and Yoshinaga, 1987; Frank, Yoshinaga and Wu, 1983). The precision of these models is modest, and they have not been widely adopted by the seafood sector. The most accurate of the empirical models has been developed by Frank (1985) for histamine formation during high-temperature storage (21.1–37.8 °C) of skipjack tuna.



(A) Predictions for 2.0 °C (dashed lines) and 4.4 °C (solid lines). (B) Predictions for 5.0 °C with 3.5% NaCl (dashed lines) and 5.0% NaCl (dotted lines). Predictions were obtained by using the Seafood Spoilage and Safety Predictor (SSSP) software (<http://sssp.dtuqua.dk>).

Regulation EC 853/2004 of the European Union (Member Organization) states that “Fresh fishery products, thawed unprocessed fishery products, and cooked and chilled products from crustaceans and molluscs, must be maintained at a temperature approaching that of melting ice” (EC, 2004a). In some countries of the European Union (Member Organization), this is interpreted as temperatures between 0 °C and +2 °C. Lightly preserved seafood with less than 6 percent salt and a pH above 5, e.g. smoked and marinated products, should be at 5 °C or less. Regulations in the United States of America specifically indicate maximum times to reach critical chill storage temperatures, but the allowed chill storage temperature of 4.4 °C is relatively high (FDA, 2011c). Storage at 2.0 °C or 4.4 °C has a markedly different effect on growth and histamine formation, as shown in Figure 13 for *M. psychrotolerans*. In the United States of America, seafood in reduced-oxygen packaging must be stored and distributed at less than 3.3 °C. This is a requirement owing to the risk of toxin formation by *Clostridium botulinum* type E (FDA, 2011d), but compared with storage at 4.4 °C the risk of histamine formation in high concentrations is also considerably lower at 3.3 °C.

Concentrations of salt above 1–2 percent NaCl reduce growth of the Gram-negative and strongly histamine-producing bacteria. For vacuum-packed cold-smoked tuna, the potential histamine formation by *M. psychrotolerans* and *P. phosphoreum* can be controlled using 5 percent water phase salt and a declared shelf-life of 3–4 weeks or less at 5 °C (Emborg and Dalgaard, 2006). As shown in Figure 13, growth and histamine formation by *M. psychrotolerans* is delayed much more by 5.0 percent water phase salt as compared with 3.5 percent water phase salt. Gram-positive bacteria including *Staphylococcus epidermis* and *Tetragenococcus muriaticus* can produce histamine at higher NaCl concentrations (Hernández-Herrero *et al.*, 1999; Kimura, Konagaya and Fujii, 2001). This may be important for fish sauce, fermented fish and salted-ripened, fish but the relative importance of these bacteria and of the activity of histidine decarboxylase produced by other bacteria prior to the mixing of fish and salt remains to be quantified.

Vacuum packing and modified-atmosphere packaging (MAP) are increasingly being used by the seafood sector. Vacuum packing reduces lipid oxidation in seafood but does not seem to delay histamine formation in fresh fish. However, MAP with gas mixtures containing carbon dioxide (CO₂) and nitrogen (N₂) can slightly delay histamine formation when high CO₂-concentrations are used. Compared with fresh MAP fish, these atmospheres delay histamine formation more efficiently for frozen and thawed products where the highly CO₂-resistant bacterium *P. phosphoreum* has been inactivated by frozen storage (Dalgaard *et al.*, 2006). For lean fish, atmospheres with high concentrations of both CO₂ and oxygen (O₂) inhibit histamine formation markedly, as shown e.g. for chilled MAP tuna (Emborg, Laursen and Dalgaard, 2005).

Growth and histamine formation by *M. psychrotolerans* can be predicted by using a new kinetic model that takes into account the effect of the initial cell concentration, storage temperature (0–25 °C), atmosphere (0–100 percent CO₂), NaCl (0–5 percent) and pH (5.4–6.5). Predictions are not highly accurate but validation studies have found an average deviation between measured and predicted times to formation of 500 mg histamine per kilogram of about 10 percent (Emborg and Dalgaard, 2008a, 2008b). To predict the effect of delayed icing/chilling of fish, and other scenarios with large variations in storage temperature, a predictive model for growth and histamine formation by both *M. morgani* and *M. psychrotolerans* has been developed (Emborg and Dalgaard, 2008b). These predictive models are included in the Seafood Spoilage and Safety Predictor (SSSP) software (available free of charge at <http://sssp.dtuqua.dk>). Development of similar predictive microbiology models for other important HPB will improve options to manage histamine formation in various fish products.

It has been shown that histamine, when formed in seafood, is relatively stable and not inactivated by freezing or heating such as normal cooking, hot-smoking or

even canning (Arnold and Brown, 1978; Taylor, 1986; Lehane and Olley, 2000; Flick, Oria and Douglas, 2001; FDA, 2011c; Kim *et al.*, 2003b). Freezing of the fish can significantly reduce the bacterial load, and it will limit the activity of decarboxylase enzymes that may have been produced prior to freezing (Kanki *et al.*, 2007).

The best ways to prevent the formation of histamine and biogenic amines in the fish industry are:

- Rapid chilling of fish immediately after death. This is particularly important for fish that are from warmer water or are exposed to warm air, and for large tuna that generate heat in the tissues of the fish following death.
- Good hygiene practices on board, at landing and during processing to avoid contamination or recontamination of the fish by bacteria capable of amino-acid decarboxylation.

Regulation EC 1441/2007 of the European Union (Member Organization) includes sampling plans ($n = 9$ and $c = 2$) and limits for critical concentrations of histamine in “fishery products from fish species associated with a high amount of histidine” where $m = 100$ mg/kg and $M = 200$ mg/kg (EC, 2007a). Samples must be taken from each batch of fish species especially of the following families: Scombridae, Clupeidae, Engraulidae, Coryfaenidae, Pomatomidae and Scombresosidae.

For “fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine” higher limits ($m = 200$ mg/kg and $M = 400$ mg/kg) are applied. The European Union (Member Organization) regulation does not include critical limits for other biogenic amines (EC, 2007a).

The United States of America uses a defect action level (m) of 50 mg of histamine per kilogram. A total of 18 fish per lot should be analysed individually or can be composited into, for example, 6 units, but then the critical limit is reduced accordingly from 50 mg/kg to 17 mg/kg (FDA, 2011c).

Examinations must be carried out in accordance with reliable, scientifically recognized methods, such as high-performance liquid chromatography (HPLC) (EC, 2007a) or fluorescent methods (AOAC, 1995).

Industry data made available to the Joint FAO/WHO Expert Meeting on Public Health Risks of Histamine and Other Biogenic Amines for Fish and Fishery Products indicated that where food business operators apply GHPs and HACCP, an achievable level of histamine in fish products is less than 15 mg/kg (based on a test method with a lower detection limit of 15 mg/kg).

3.2.3 Viruses (Iddya Karunasagar and David Lees)

Viruses are very small micro-organisms (15–400 nm) that consist of a nucleic acid (DNA or ribonucleic acid [RNA]) associated with proteins and, in some cases, they may also have a lipid bilayer membrane (or envelope). Viruses are obligatory intracellular pathogens and cannot multiply outside their host cells, although they may survive for long periods outside the host cells. Thus, viruses do not replicate in food or water. Viruses can infect all major groups of organisms from bacteria to mammals. Viruses are classified according to the nature of their genome (DNA or RNA, single stranded or double stranded, segmented or non-segmented, linear or circular and, in the case of single stranded RNA viruses, whether it can function as messenger RNA [mRNA]) and, in addition, their structure (symmetry, enveloped or not, number of capsomeres). Viruses cause a number of diseases in humans ranging from the common cold to serious illnesses such as rabies and HIV/AIDS. Viruses are abundant in nature and most are not pathogenic to humans. There are millions of virus-like particles in a millilitre of seawater, and they are a major cause of mortality in bacteria and plankton. Thus, viruses play a very important role in nutrient and energy cycles in the marine environment (Suttle, 2007). However, these viruses are not pathogenic to humans.

Food-borne viruses are derived from the human gastrointestinal tract, and their presence in water and food is a result of contamination with sewage, poor hygiene or contamination by food handlers.

TABLE 30
Groups of viruses implicated in food and waterborne illnesses

Virus	Type and characters	Illness	Association with seafood
Norovirus	(+) ssRNA, non-enveloped with icosahedral symmetry	Epidemic gastroenteritis	Bivalve molluscs
Astrovirus	(+) ssRNA, non-enveloped with icosahedral symmetry	Gastroenteritis	Epidemiological evidence limited, but shellfish associated outbreak reported
Hepatitis A virus	(+) ssRNA, non-enveloped with icosahedral symmetry	Inflammation of liver, hepatitis	Bivalve molluscs
Enteroviruses (e.g. poliovirus, coxsackie A, B)	(+) ssRNA, non-enveloped with icosahedral symmetry	Poliomyelitis, meningitis, encephalitis	No seafood associated outbreak reported. Detection in shellfish reported
Rotavirus	ds RNA, non-enveloped with icosahedral symmetry	Gastroenteritis	mainly water-borne
Adenovirus	ds DNA, non-enveloped with icosahedral symmetry	Respiratory, eye and gastrointestinal infections	No seafood associated outbreak reported. Detection in shellfish reported

Source: Greening (2006).

Although a number of viral groups (Table 30) have been detected in shellfish, clear epidemiological links with seafood exist only for norovirus and hepatitis A virus. Astrovirus has also been reported as an aetiological agent in a limited number of shellfish-associated outbreaks. However, reported outbreaks may underestimate the actual burden of infection as food-borne viruses are seldom the cause of mortality and many infections may not be reported, owing to lack of investigation to confirm illness. Frequently, in outbreaks, a viral aetiology may not be confirmed as, for practical purposes, neither norovirus nor hepatitis A viruses can be cultured in cell lines, and facilities may not be available for detection of culturable viruses in most clinical diagnostic laboratories. With the advent of molecular techniques, it is possible to detect non-culturable viruses, but these tests require sophisticated laboratories and expertise. Therefore, a large number of cases go undiagnosed, and illness caused by norovirus is not notifiable even in developed countries (Richards, 2006) and only large outbreaks may be investigated and reported. Thus, published epidemiological data are of limited value in establishing the true burden of infection caused by viruses in molluscan shellfish.

3.2.3.1 *Norovirus*

This virus was first reported in an outbreak of gastroenteritis in a school in Norwalk, Ohio, the United States of America (Kapikian *et al.*, 1972) and subsequently called Norwalk-like virus (NLV) or small round structured viruses (SRSVs). This virus has been characterized as belonging to the family Calciviridae, which has two genera: *Norovirus* and *Sapovirus*. This family is characterized by the presence of a single major structural protein making up the capsid and 32 cup-shaped depressions (from which the name Calciviridae is derived, calyx in Latin meaning cup) on the surface of the virion in an icosahedral symmetry. The viruses are small (30–35 nm in diameter) and the genome is a positive single-stranded non-segmented RNA. Based on the nucleotide sequence of highly conserved regions of the genome, such as the RNA-dependent RNA polymerase and capsid gene, the norovirus is grouped into five (I–V) genogroups (Karst *et al.*, 2003). The noroviruses affecting cattle are in genogroup III, and murine viruses in genogroup V (Busea and Rodriguez-Diaz, 2006). Human noroviruses have not been cultured so far. Sapoviruses are most commonly associated with diarrhoea in infants and children. The transmission of Sapoviruses is likely to be from person

to person and food-borne transmission is rare, while seafood has so far been not implicated (Greening, 2006).

Noroviruses are transmitted primarily by the faecal–oral route through contaminated water or food, but subsequent person-to-person spread occurs frequently. The virus is highly infectious and 10–100 viral particles may cause clinical symptoms (Caul, 1996). It has been reported to affect all age groups. The clinical symptoms appear after an incubation period of 1–4 days and may include nausea, vomiting, abdominal pain, diarrhoea, and fever generally, followed by complete recovery (Lees, 2000). The illness may last 2–3 days. Persons with clinical illness may shed $> 10^6$ viral particles per millilitre of stool. One of the characteristic symptoms has been projectile vomiting, which has been reported to contribute to secondary spread through droplet infection (Greening, 2006). Viruses can be shed in high numbers in the vomitus (D'Souza, Moe and Jaykus, 2007). This secondary spread makes estimation of illness attributed to food very difficult. Attack rates have been reported to be 50–70 percent or even higher in some cases (Greening, 2006). Viruses are shed in stools before symptoms occur and may continue for three weeks after recovery (D'Souza, Moe and Jaykus, 2007). Host susceptibility may vary depending on genetic factors and acquired immunity. Human volunteer studies indicate that individuals lacking H type 1 histo-blood group antigen were unaffected even when exposed to high doses, and subsequent studies have indicated that this antigen serves as receptor for norovirus binding. About 20 percent of Europeans are negative for this antigen, and in Asian and African ethnic groups this proportion has been reported to be even higher (D'Souza, Moe and Jaykus, 2007). Moreover, in human challenge studies, individuals of blood group O were found to be more susceptible than those with A or B groups (D'Souza, Moe and Jaykus, 2007). Acquired immunity appears to be short-lived with human challenge studies indicating that those who become ill on primary exposure are not susceptible when rechallenged at 6–14 weeks, but they do become ill when rechallenged at 24–42 months (D'Souza, Moe and Jaykus, 2007).

A variety of foods including shellfish, meats, bakery products and raspberries have been implicated in food-borne transmission of norovirus (Greening, 2006). Epidemiological investigations have been greatly assisted by the use of DNA sequencing techniques for genotyping noroviruses. An outbreak across six states in the United States of America could be related to oysters harvested from a single area in 1993 (Dowell *et al.*, 1995). Fankhauser *et al.* (2002) reported that 93 percent of 284 non-bacterial gastroenteritis outbreaks in the United States of America were due to norovirus and that contaminated food was the vehicle of infection in 57 percent of these. In Europe, 85 percent of nonbacterial gastroenteritis, between 1995 and 2000, was attributed to noroviruses (Lopman *et al.*, 2003). According to United States FoodNet data (CDC, 2006b), noroviruses accounted for 52 percent of the food-borne outbreaks for which aetiology could be confirmed in 2004. In the period 1998–2002, norovirus caused 30 percent of the food-borne disease outbreaks (657 outbreaks and 27 171 cases) of known aetiology in the United States of America (Lynch *et al.*, 2006). Several shellfish-associated outbreaks have been reported. An outbreak involving 472 cases of gastroenteritis due to oyster consumption in Louisiana resulted in the closure of 25 percent of 250 000 acres (100 000 ha of shellfish beds with an estimated loss of US\$5.5 million for 500 licensed oyster harvesters (Richards, 1985). Overboard dumping of sewage by oyster harvesters has been reported to be the cause of contamination in several outbreaks (Lees, 2000). A large outbreak involving more than 2 000 cases linked to oyster consumption occurred in Australia in 1978 (Murphy *et al.*, 1979). In Japan, norovirus was implicated in 53 out of 80 oyster-associated outbreaks in the period 1984–1987 (Sekine *et al.*, 1989). Several shellfish associated outbreaks have been reported in Europe (Richards, 2006). Outbreaks have also occurred through consumption of oysters that were purified by depuration, suggesting that this process

is inadequate to protect against viral infections (Lees, 2000). A reduction of only 7 percent in norovirus was seen after depuration treatment of 48 h (Schwab *et al.*, 1998). Noroviruses have been shown to bind specifically to shellfish tissue receptor sites (digestive ducts, midgut, main and secondary ducts, and tubules) (Le Guyader *et al.*, 2006a), and this might explain virus retention after depuration.

In addition to contamination at source, food handlers may also be a source of contamination with noroviruses. A study in the Netherlands indicated that norovirus shedding could be detected in 5.2 percent of individuals without complaints of gastroenteritis and 19 percent in asymptomatic individuals in an outbreak setting (Vinje, Altena and Koopmans, 1997; De Wit *et al.*, 2001).

Inability to culture human norovirus has hampered studies on factors affecting virus survival in the environment and susceptibility to physical and chemical agents. Feline calicivirus has been used as a model in inactivation studies. However, as this is a respiratory virus, there are concerns about applicability to enterically transmitted viruses. Feline calicivirus was not efficiently inactivated on environmental surfaces or in suspension by 1 percent anionic detergents, quaternary ammonium (1:10), hypochlorite solution with < 300 ppm free chlorine, or ethanol at less than 50 percent or more than 80 percent (Diuzer and Koopmans, 2006). A contact time of 10 min with sodium hypochlorite containing 500 ppm available chlorine was required to bring about a 3 log₁₀ or higher reduction in infectivity and at 1 000 ppm, contact time could be 1 min. Commercial hand-rub agent containing 70 percent ethanol caused 1.42 log₁₀ reduction at a contact time of 20 s (Sattar and Bidawid, 2006). Pasteurization (70 °C for 2 min) would inactivate the virus. Norovirus is resistant to refrigeration, freezing and low pH. An outbreak linked to orange juice (pH ~3.5) and human volunteer infection after incubation at pH 2.7 for 3 h show acid tolerance of noroviruses.

3.2.3.2 *Hepatitis A virus*

Hepatitis A virus (HAV) is a member of the genus *Hepatovirus* belonging to the family Picornaviridae (D'Souza, Moe and Jaykus, 2007). HAV is a small (27–32 nm) non-enveloped virus with icosahedral symmetry and a positive-sense, single stranded RNA genome of 7.5 kb in size. There are two strains or biotypes: human HAV and simian HAV. The human strain infects all species of primates, while the simian virus infects green monkeys and cynomolgus monkeys (Greening, 2006). Of the seven genotypes recognized, four (genotypes I–III and VII) infect humans. Notwithstanding the genetic variation, human HAV comprises a single serotype (D'Souza, Moe and Jaykus, 2007). Although HAV can be cultured in a number of primate cell lines such as African green monkey kidney cells (BSC-1), foetal rhesus monkey kidney cells (FRhK-4 and FRhK-6) and human fibroblasts, wild-type strains are difficult to culture. The virus is slow-growing and requires three weeks for *in vitro* growth. The virus may not produce cytopathic effects in cell cultures, and viral antigen in cell cultures can be detected by immunofluorescence. Therefore, it is difficult to identify the virus in clinical or food samples by culture alone.

The primary route of transmission is by the faecal–oral route, but the virus can also be transmitted by person-to-person contact. In developing countries, where the virus is endemic, it is estimated that more than 90 percent of children are infected by the age of six years and, often, the infections are asymptomatic (Greening, 2006). The incubation period is long (2–7 weeks) with an average of 28 days, and this makes tracking the source of infection difficult. Four phases of clinical features are recognized: an asymptomatic phase, in which the virus replicates in the host; a preicteric phase, characterized by anorexia, nausea, vomiting and malaise; an icteric phase characterized by jaundice and hepatosplenomegaly; and a convalescent phase (D'Souza, Moe and Jaykus, 2007). Although icteric disease is rare (< 10 percent of those infected) in children below 6 years, it occurs in 40–50 percent of older children and 70–80 percent of infected

adults. The illness may last several weeks, but is self-limiting with a mortality rate of about 0.01 percent (D'Souza, Moe and Jaykus, 2007). Two weeks prior to the onset of jaundice, the virus is present in the blood ($\sim 10^4$ virions/ml) and viremia may last 2–4 weeks. Faecal shedding of the virus ($> 10^6$ particles/g) occurs during the second week of incubation and lasts throughout the clinical phase. The virus may be detectable in stools for three months after acute illness. As the virus is shed even before clinical symptoms appear, infected individuals may unknowingly spread the virus by handling food at this stage (Greening, 2006). Immunity following infection is lifelong, and a killed virus vaccine is available, which could be used by the food industry to immunize food handlers.

Outbreaks of illness due to HAV have been reported from several countries. In many developing countries, children below six years contract infection that is often asymptomatic in this age group and develop immunity. In developed countries, adults may not have been exposed as children and are thus susceptible to illness. In the United States of America, hepatitis is a notifiable disease; hence, records of disease burden are available. Although the average number of cases notified to CDCs in the period 1980–2001 is 25 000 cases/year, the actual incidence is thought to be 10 times higher (Fiore, 2004). However, most infections are derived from contact with infected individuals and food-borne cases account for only a small percentage. Infections have been recorded throughout the year without any noticeable seasonality. The largest reported outbreak occurred in China in 1988, affecting about 300 000 people, who had consumed partially cooked clams from a sewage-affected area (Halliday *et al.*, 1991). A variety of shellfish have been implicated in different countries: oysters in Australia (Conaty *et al.*, 2000) and Brazil (Coelho *et al.*, 2003), mussels in Italy (Crocì *et al.*, 2000) and clams in Spain (Bosch *et al.*, 2001). In Japan, an outbreak linked to a sushi bar was reported (Takeuchi *et al.*, 2006). In a large outbreak affecting 882 people in Italy in 2004, shellfish harvested from different areas but kept alive in seawater in a contaminated area were found to be the source of infection (Pontrelli *et al.*, 2008). Outbreaks associated with oysters, cockles and mussels have been reported in Ireland and in England and Wales, the United Kingdom of Great Britain and Northern Ireland (Richards, 2006).

Hepatitis A virus can survive in the environment for long periods. In dried faeces, HAV remained infectious for 30 days when stored at 25 °C and 42 percent relative humidity (Hollinger and Ticehurst, 1996).

Hepatitis A virus reaching the environment through sewage can survive for a long time in water and sediment. In seawater, 90 percent inactivation of HAV occurred after 671 days at 4 °C and after 25 days at 25 °C (Papafraqkou, D'Souza and Jaykus, 2006). Detection of viral RNA in marine sediments has been reported from Spain and France (Bosch and Pinto, 1992; Le Guyader *et al.*, 1994). Infectious virus was detected in oysters 3 weeks after contamination and viral RNA was detected up to 6 weeks (Kingsley and Richards, 2003). Hepatitis A virus appears resistant to depuration with outbreaks related to depurated shellfish occurring (Richards, 2006). In Italy, HAV was detected in 20 percent of non-depurated mussels, 11 percent of depurated mussels and 23 percent of mussels in various markets (Chironna *et al.*, 2002). Bacterial indicators of faecal contamination of shellfish are inadequate to indicate the presence of viruses including HAV. In Italy, HAV was detected in 13 out of 36 mussels harvested from areas meeting European bacteriological standards (Crocì *et al.*, 2000).

Hepatitis A virus survived freezing in strawberries for up to 2 years, and remained infectious at pH 1.0 for 5 h (D'Souza, Moe and Jaykus, 2007). In mussels, HAV retained infectivity following simulated commercial marinating and exposure to acid conditions (pH ~ 3.75) at 4 °C over 4 weeks (Hewitt and Greening, 2004). Studies with steamed crabs revealed that complete inactivation of HAV at 100 °C took 4–6 min, but in mussels, 100 °C for 2 min completely inactivated HAV (Crocì *et al.*, 1999). In

contaminated cockles immersed in water at 85 °C, 90 °C or 95 °C or steamed for 1 min, only partial inactivation of HAV was noted, but when internal temperature of shellfish reached 85 or 90 °C for 1 min, HAV was inactivated. The European Union (Member Organization) heat processing recommendation for bivalve molluscs – an internal temperature of 90 °C for 1.5 min – has been reported to be adequate for HAV and also feline calicivirus (D'Souza, Moe and Jaykus, 2007). HAV is resistant to irradiation – a radiation dose of 3 kGy could reduce HAV titre by less than 2 log₁₀ (D'Souza, Moe and Jaykus, 2007). A hydrostatic pressure of 450 MPa and 275 MPa for 5 min (Kingsley *et al.*, 2002) inactivated HAV suspended in tissue culture medium and these pressures were also effective in oyster meats (Calci *et al.*, 2005).

As infected individuals shed large numbers of viruses in stools, they could transmit the virus by handling food. In human volunteers, the laboratory-adapted strain had a half-life of from 5.5 to 7 h on fingers (Bidawid, Farber and Sattar, 2000). About 25 percent virus transfer occurred during a casual (10 s) contact between contaminated and clean surfaces such as finger pad to metal disc and vice versa (Sattar *et al.*, 2000). Disinfection of finger pads with 75–62 percent ethanol led to virus recovery of 24–64 percent (Bidawid, Farber and Sattar, 2000). Thus, complete virus removal could not be achieved. Hepatitis A virus on stainless steel or polyvinyl chloride surfaces survives a majority of disinfectant treatments. Of about 20 formulations tested, only 2 percent glutaraldehyde, quaternary ammonium compound containing 23 percent hydrochloric acid, sodium hypochlorite with free chlorine > 5 000 ppm showed virucidal activity (Papafragkou, D'Souza and Jaykus, 2006).

3.2.3.3 *Methods for detection of viruses in bivalve molluscs*

In the last decade, considerable progress has been made in detection methods for norovirus and HAV in molluscan shellfish. As these viruses cannot be cultured, all methods currently proposed for routine detection are based on virus genome detection using PCR. This is the only method with the demonstrated sensitivity to pick up the low virus levels found in food. Bivalve molluscs present a challenging target; methods need to be capable of extracting low levels of contaminating virus and presenting them in a non-inhibitory extract to a sensitive PCR assay. Dissected bivalve digestive organs are generally used as the starting material for virus extraction. Digestive tissues comprise approximately 10 percent of the body mass of the bivalve but the large majority of the contaminating virus, possibly due to specific adherence mechanisms (Le Guyader *et al.*, 2006b). This enriched tissue needs to be further processed to recover virus and/or its RNA and to remove inhibitors prior to reverse transcription PCR. Recently, most work has focused on real-time PCR which has significant technical advantages, including inbuilt probe confirmation and the potential for quantitation and standardization (Jothikumar *et al.*, 2005; Loisy *et al.*, 2005; Costafreda, Bosch and Pintó, 2006). Norovirus strains causing human infections are classified into two genogroups: GI and GII. Both genogroups are common contaminants of sewage and should be targeted. The genetic diversity of norovirus strains dictates the need for careful selection of PCR reagents. The ORF1-ORF2 junction region (Kageyama *et al.*, 2003) is highly conserved and used by most workers (Jothikumar *et al.*, 2005; Loisy *et al.*, 2005). In non-endemic areas, HAV may be a relatively rare contaminant in bivalve shellfish because of low levels in the community. However, the disease is more severe than norovirus and the consequences of an outbreak can be dramatic (Bosch *et al.*, 2001; Shieh *et al.*, 2007). Therefore, laboratories should have the capability to detect both norovirus and HAV. For HAV, real-time PCR assays targeting the highly conserved 5' non-coding region have been shown to be both sensitive, cross-reactive and robust (Costafreda, Bosch and Pintó, 2006). Finally, it is necessary to consider the selection of assay controls carefully. The known inhibitory potential of bivalves and the public health significance of low virus levels (Sánchez *et al.*, 2002; Le Guyader *et*

al., 2003, 2006b; Costafreda, Bosch and Pintó, 2006) dictate the use of both sensitive real-time PCR inhibition controls and the use of a process control to ensure adequate virus recovery. To facilitate interlaboratory comparison, a requirement is to report results in meaningful units, such as virus RNA genome copies per weight of material tested. However, despite these advances, virus testing is currently not incorporated as an element of regulatory controls within, for example, the major markets of the European Union (Member Organization) or the United States of America. A major factor is the current absence of any standardized and validated methods with the demonstrable performance necessary to both protect public health and avoid trade disputes. Within the European Union (Member Organization), a network of specialist reference laboratories, participating in the European Committee for Standardization (CEN), are working towards the development of a standard method for detection of human pathogenic viruses in foods (including bivalve molluscs), which may help resolve some of these issues. However, research studies in several countries suggest that virus is commonly detected in commercially produced bivalves using PCR (Bosch, Pinto and Le Guyader, 2009). An unknown factor is whether this represents the detection of fully infectious virus that would cause illness following shellfish consumption.

3.2.3.4 *Risk management strategies for viruses in bivalve molluscs*

Contamination of shellfish with human pathogenic viruses occurs through sewage contamination. Collaboration between environment/wastewater treatment authorities and public health and food safety authorities is required to prevent contamination of shellfish-growing areas. Currently, the risk management tool used in management of shellfish safety with respect to bacterial pathogens is to regulate shellfish production areas based on levels of the faecal indicator bacteria, faecal coliforms/*E. coli*. Most countries with bivalve mollusc production (including the United States of America and the European Union [Member Organization]) require monitoring of areas, followed by their classification or grading according to faecal contamination risk. The grading determines the risk management measures that must be applied prior to placing products on the market for consumption. Table 31 summarizes the requirements for the markets of the United States of America (FDA, 2007) and the European Union (Member Organization) (EC, 2004b). An essential first step, prior to setting up a monitoring programme, is to survey the faecal pollution inputs, and their potential circulation within the production area, so that monitoring programmes and risk management measures can be scientifically based. This “sanitary survey” is a requirement of regulations in both the United States of America and the European Union (Member Organization). Risk management measures used for bivalves include harvesting only during periods of good water quality (according to an agreed management plan), depuration (self-purification in tanks), relaying in good-quality areas, heat processing (using steam or by immersion in water), high-pressure treatment, and temporary or more long-term production area closure. These measures vary in their effectiveness for viruses. Heat processing can be very effective if performed correctly (EC, 2004a). In the United Kingdom of Great Britain and Northern Ireland, following the introduction of revised criteria (raising core mollusc temperatures to 90 °C for 90 s), hepatitis outbreaks from cockles harvested in the estuary of the Thames River were brought under control (Lees, 2000). However, for products marketed live, depuration, relaying and harvest area management, while effective at controlling bacterial infections (such as salmonellosis and typhoid), have been less effective for viruses. Depuration is a widely used commercial processing option. However, both epidemiological and laboratory studies show that the depuration times and conditions currently used are inadequate to remove viruses (Lees, 2000). Recent studies suggest high-pressure treatment may be effective for reducing viral loads (Calci *et al.*, 2005). The universally accepted measure of acceptable quality of products placed on the market is < 230 *E. coli* (or < 300 faecal

coliforms) per 100 g of shellfish flesh. However, there are a number of examples where products have been produced in accordance with the sanitary requirements but have still caused viral outbreaks (Lees, 2000; Croci *et al.*, 2000; Bosch, Pinto and Le Guyader, 2009). Alternate indicators such as coliphages or adenovirus have been suggested (Doré, Henshilwood and Lees, 2000; Formiga-Cruz *et al.*, 2003), but none has been accepted anywhere. Most activity now focuses on the development of specific viral standards for norovirus and HAV. However, the current non-availability of reliable molecular methods that have undergone interlaboratory calibration for detection of viruses and the lack of information on viability or infectivity of viruses detected by molecular methods are some of the constraints in this area. Despite this, some countries have already introduced virus certification requirements for imports, and further developments can be expected.

TABLE 31

Summary of sanitation requirements in the European Union (Member Organization) and the United States of America for live bivalve mollusc production areas

Risk management measure required	United States of America FDA classification	Microbiological standard per 100 ml water	European Union (Member Organization) classification	Microbiological standard per 100 g shellfish
Non-required	Approved	GM ¹ < 14 FCs ² and 90% ³ < 43 ⁴ FCs	Category A	all samples < 230 <i>E. coli</i>
Depuration or relaying	Restricted	GM < 88 FCs and 90% < 260 ⁴ FCs	Category B	90% ⁵ < 4 600 <i>E. coli</i>
Relaying over a long period	–	–	Category C	all samples < 46 000 <i>E. coli</i>
Harvesting prohibited	–	above levels exceeded	–	above levels exceeded

¹ Geometric mean.

² Faecal coliforms.

³ Samples must have a 90%-ile compliance with the standard.

⁴ The upper limit varies marginally according to the accuracy of the method used.

⁵ Transitional arrangement under EC 1666/2006.

3.2.4 Parasites (Darwin Murrell and Anders Dalsgaard)

Fish-borne zoonotic parasites are prevalent in many regions of the world and are among the most important of all zoonotic parasites infecting humans (WHO, 1995, 2004a; Keiser and Utzinger, 2005). The number of people currently infected with these parasites may exceed 20–30 million, with the number of people at risk worldwide estimated at more than half a billion (WHO, 2004a; Keiser and Utzinger 2005; Muller, Schmidt and Melhorn, 2007) (Table 32). For example, in Asia, there are about 1.5 million people in the Republic of Korea, 6 million people in China, and more than 5 million in Thailand infected with the liver flukes *Clonorchis sinensis*, *Opisthorchis viverrini* or *O. felineus* (Chai, Murrell and Lymbery, 2005). The recognition of the public health significance of these zoonoses (and of their links to poverty and cultural traditions, to intensification of agriculture, to environmental degradation, and of the lack of proven procedures and tools for their control) is increasing (WHO, 1995, 2004).

Although the zoonotic parasites of fish represent only a minority of the many parasite species that infect fish, they are a widespread and diverse group. Most of the zoonotic species are normally parasites of non-human land and aquatic mammals or fish-eating birds; people become infected by eating raw or improperly prepared fish. Fish-borne parasites are primarily helminths, and include species of nematodes (round worms), cestodes (tapeworms) and trematodes (flukes). They are found in both marine/brackish-water and freshwater wild and cultured fish. In all the important species, it is a larval stage present in the fish host that is transmitted to a suitable final (definitive) host, in which full development to the reproducing adult stage occurs.

However, for some parasite species, the larval stage does not mature in the human host (i.e. the nematodes *Anisakis* and *Gnathostoma*). Remaining in a larval stage, they can migrate through the host's tissues causing pathological damage. Importantly, fish-borne parasite infections in people often exist as a multiple species complex (Dung *et al.*, 2007), because they have common transmissions modes that are favoured by well-entrenched cultural traits, particularly food behaviour, for example, a fondness for raw or improperly cooked, cured or pickled fish and fish products.

TABLE 32

Estimates of numbers of global human infections with major fish-borne parasites

Helminth species	Numbers (millions)	References
TREMATODA		
Liver flukes <i>Clonorchis sinensis</i> <i>Opisthorchis</i> spp., others	17	WHO, 1995, 2004a
Small intestinal flukes ¹	no estimates, this group only recently recognized as widely distributed and common	WHO, 1995; Chai, Murrell & Lymbery, 2005; Chai, 2007
CESTODA		
<i>Diphyllobothrium</i> spp. ²	9–20	Von Bonsdorff, 1977; Muller, 2001
NEMATODA		
<i>Anisakis simplex</i> <i>Pseudoterranova decipiens</i>	0.33	Ishakura <i>et al.</i> , 1998; Lymbery & Cheah, 2007

¹ A collective title for species of flukes belonging to the Heterophyidae, Echinostomatidae, Neodiplostomidae and Plagiorchiidae.

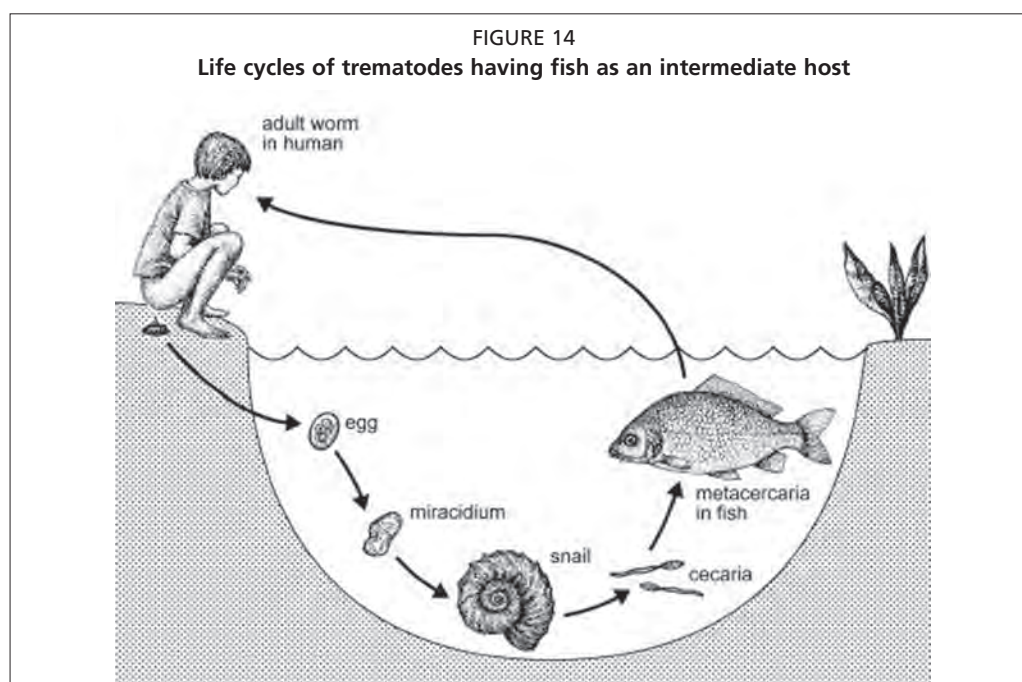
² Six or more species implicated in human infections.

Source: Modified from Murrell and Crompton (2009).

3.2.4.1 Trematode species

The trematodes or flukes are non-segmented flatworms (Platyhelminthes), characterized by possession of oral, and usually, ventral suckers and they are hermaphroditic. Their life cycles require one or more intermediate hosts (a molluscan host is universal), and, in the case of fish-borne flukes, a second intermediate fish host (Figure 14). The range of fish hosts encompasses more than ten species of freshwater and brackish-water/marine species (Chai, Murrell and Lymbery, 2005). The cercarial stage, which is shed from an infected mollusc (snail), invades the fish and encysts, chiefly in muscles, less frequently under the scales, fins or gills, and transforms into an encysted metacercaria. The fish-borne zoonotic flukes can be divided into two major groups, based on the site of infection in the definitive host: liver/bile duct, or intestine.

Liver flukes: The essential features of liver flukes' distribution, life cycle, epidemiology and risk factors associated with their transmission are shown in Table 33. The major liver fluke species, *Clonorchis sinensis*, *Opisthorchis viverrini* and *O. felinus* are closely related and share many biological traits. *C. sinensis*, is widely distributed in East Asia (Chai, Murrell and Lymbery, 2005). The number of people infected currently in the Republic of Korea is estimated at about 1.5 million (Chai, Murrell and Lymbery, 2005) and in China, the prevalence of *C. sinensis* was reported as 0.4 percent among almost 1.5 million people examined; based on this, the number of infected people in China may be about 6 million (Xu *et al.*, 1995). In Viet Nam, clonorchiasis is endemic, especially in the Red River Delta area in the north of the country (De *et al.*, 2003). *O. viverrini* affects 10 million people or more in Thailand, the Lao People's Democratic Republic and Viet Nam (Yossepowitch *et al.*, 2004); *O. felinus* is reported frequently in Eastern and Southeastern Europe and the Asiatic parts of the Russian Federation, but there are few prevalence data available.



Source: Huss, Ababouch and Gram (2004).

TABLE 33

Features of the distribution, biology, transmission and public health risks of fish-borne trematode infections

Parasites Trematodes (flukes)	Major geographic distribution	Biology and transmission features	Risk factors
LIVER FLUKES			
<i>Clonorchis sinensis</i>	Asia, Japan, China, the Republic of Korea, Pacific region, Near East, United States of America.	Adult worms of three species in fish-eating mammals, including humans, dogs, cats, pigs and rats.	Infection of definitive host through ingestion of raw or insufficiently cooked, pickled or smoked infected fish.
<i>Opisthorchis viverrini</i>	Asia Thailand, Lao People's Democratic Republic, Viet Nam, Cambodia.	First intermediate host: snails, in which parasite reproduces asexually, and emerges as a swimming cercaria stage, seeking freshwater fish, especially carps, which are important in aquaculture. Cercariae invade muscles, viscera, gills, fins and scales and encyst (metacercaria).	Eating pickled or raw fish at parties or restaurants involving alcohol is especially risky for adults.
<i>O. felineus</i>	Central and Eastern Europe, Turkey, eastern Siberia.		Allowing domestic animals to eat raw fish increases risk of establishing reservoir hosts. Use of human and animal waste for pond fertilization is an important risk for fish. Failure to control snails in aquaculture systems is also an important risk factor.
INTESTINAL FLUKES			
Examples: <i>Haplorchis</i> <i>Heterophyes</i> <i>Metagonimus</i> <i>Heterophyopsis</i> <i>Sticidora</i> <i>Stellantchasmus</i> <i>Echinochasmus</i>	Zoonotic species are distributed worldwide, especially Asia, Central Asia (India), Near East	Adult worms in fish-eating birds and humans and reservoir hosts such as dogs, cats, pigs, rats, various wild animals. Asexual multiplication occurs in snails, which yields swimming cercariae that seek out intermediate hosts (usually fish). Cercaria penetrates gills, viscera, fins, scales and muscles to encyst (metacercaria). At least 45 genera of freshwater and marine/brackish-water fish are susceptible, many of which are important in aquaculture.	For humans and reservoir hosts, consumption of raw, insufficiently cooked, pickled or smoked fish are most important risks. Parties accompanied by alcohol may be responsible for frequent gender difference in prevalence in humans. Use of human and animal waste for fish pond fertilization represents a high risk for fish infection.

Source: Modified from Murrell and Crompton (2009).

The round or oval metacercaria (larvae), measuring 0.13–0.14 mm by 0.09–0.10 mm, can be found in more than 100 species of freshwater and brackish-water fish belonging to 13 families, especially the Cyprinidae, which are important in aquaculture (WHO, 1995). In the definitive host (including humans), the metacercariae excyst in the duodenum and migrate to the common bile duct and then to the extrahepatic and intrahepatic bile ducts. The metacercariae grow to the adult stage in about 4 weeks after infection; the adult worm is flat, elongate, lanceolate, and 5.5–9.6 mm long and 0.8–1.7 mm wide (Rim, 1986). The hepatic lesions and clinical manifestations in infected people are similar for all the liver fluke infections. Complications, such as pyogenic cholangitis, biliary calculi, cholecystitis, liver cirrhosis, pancreatitis and cholangiocarcinoma, are often associated with infection (Sripa, 2003). High incidences of cholangiocarcinoma, based on both necropsy and liver biopsy data, have been reported for *O. viverrini* in northeast Thailand, where cholangiocarcinoma is estimated to occur in 129 per 100 000 males and in 89 per 100 000 for females, compared with 1–2 per 100 000 persons in western countries (Vatanasapt *et al.*, 1990). The severity of the pathology is associated with both intensity and duration of infection and the location of the lesions (Rim, 1986).

The major risk for acquiring liver flukes in endemic areas is related to the custom of eating raw fish. The morning congee (rice gruel) with slices of raw freshwater fish (southern China and China, Hong Kong SAR) or slices of raw freshwater fish with red pepper sauce (the Republic of Korea) are examples of major dietary sources of *C. sinensis* infection (Murrell and Crompton, 2009). In northeast Thailand and the Lao People's Democratic Republic, “Koi pla”, a popular raw fish dish, is an important food source of infection with *O. viverrini*.

The most important risk factor for infection of fish is the exposure to waterbodies containing susceptible species of hydrobid snails to faeces from infected humans and other reservoir hosts (e.g. dogs, cats, pigs). In many countries, because of the cost benefits to aquaculture, human and animal faeces are utilized as pond fertilizer. Although the prevalence of infection in a snail population can be as low as 0.08 percent, even in highly endemic areas, this is sufficient to maintain the life cycle because snails infected with *C. sinensis* may release an average of 788 cercariae per snail daily, with a maximum 5 840 cercariae per snail (Rim, 1982). Although the infection rates of snails with *O. viverrini* are similar (0.083–1.6 percent), this level is sufficient to maintain endemicity (Kaewkes, 2003). Both the prevalence and intensity of infection of the fish with metacercariae can be very high; often, 94–100 percent of fish examined can be infected with zoonotic metacercariae (Ooi *et al.*, 1999).

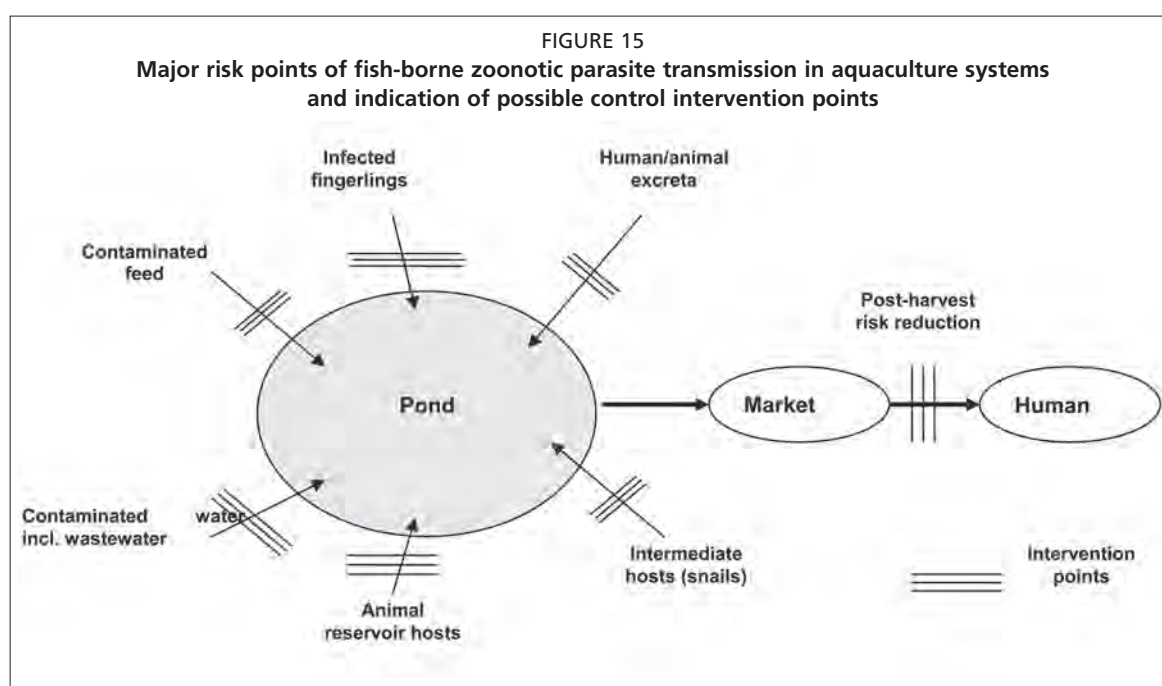
Diagnosis of liver fluke infections can be made by faecal examination, such as the Kato-Katz technique (Hong *et al.*, 2003). However, the eggs must be differentiated from those of intestinal flukes (see below) which are very similar (Ditrich *et al.*, 1992), a task that requires considerable training and experience. More recently, molecular techniques have been reported that can differentiate liver flukes from other trematode eggs (Muller, Schmidt and Melhorn, 2007; Traub *et al.*, 2009). Molecular (PCR) tools for detecting liver fluke metacercariae in fish have also been developed (Parvathi *et al.*, 2008).

Technical reports of the WHO on trematode infections have described in detail various strategies for the control of liver (and intestinal) flukes (WHO, 1995, 2004a). Currently, the major strategies for community prevention and control include faecal examination and treatment of individual human cases with praziquantel (25 mg/kg, 3 times daily, for 2–3 days), health education to promote the consumption of properly cooked fish, and environmental sanitation through the building and use of latrines in endemic areas. The WHO (2004a) recommends mass chemotherapy of people at risk in endemic areas as the most practical and immediately effective control strategy. Mass chemotherapy with praziquantel (40 mg/kg in a single dose) is highly efficient

and generally feasible to distribute. Because of the lack of control programmes that have been followed over time, the long-term effectiveness of this approach has not been evaluated yet (Murrell and Crompton, 2009). One potential weakness may be the failure to take into account animal reservoir hosts (e.g. dogs, cats and pigs) living in the location that could sustain the fluke life cycle in the absence of eggs from human hosts (Anh *et al.*, 2009).

Control of liver fluke (and intestinal fluke) infections in cultured fish has not been intensively studied to date. However, the basic elements for intervention are shown in Figure 15 and include:

- Prevent fluke eggs from contaminating the waterbodies.
- Treat household members and their domestic animals (potential reservoir hosts) to remove source of egg contamination.
- Remove or control vector snail species from the fish ponds.



Source: Modified after WHO (1995).

One HACCP-based control trial on *O. viverrini* in pond-reared fish in Thailand has been reported (Khamboonraung *et al.*, 1997). Although in the first year the effort achieved a significant reduction in fish infection, no subsequent reports on the sustainability of these control interventions have been made; therefore, the long-term effectiveness remains unknown.

Likewise, there has been only limited research on methods to inactivate metacercariae in fish and fish products (Table 34). The methods evaluated are those associated with preservation such as temperature, pH and water activity (salting).

More work should be undertaken to gain a better knowledge of the necessary heat treatment needed to inactivate trematodes in fish. Freezing provides an effective mean of inactivating most parasites in raw fish, but the data on metacercariae of *C. sinensis* (Fan, 1998) indicates that 7 days at -20°C had no inhibitory effect on their viability in naturally infected fish. In contrast, based on the work of Fattakhov (1989), the Ministry of Health of the then Soviet Union recommended (in 1990) holding fish at -28°C for 32 h or at -40°C for 7 h to inactivate the trematode *O. felinus* in fish (Table 34). *O. viverrini* was virtually unaffected when stored in saline solution at 4°C for 5 weeks (Sithithaworn *et al.*, 1991). The differences observed in the experiments reported in

Table 34 may reflect either or both the differences in the methods employed by the investigators, especially the methods used to evaluate the viability of metacercariae (microscopic-based examination of the ability of metacercariae to excyst versus the more reliable animal infection method, where metacercariae are fed to animals and their ability to cause infection is assessed by the identification of adult flukes in necropsied animals).

TABLE 34
Preservative parameters necessary to inactivate trematodes

Preservative parameter	Parasite	Process variable	Time	Reference
Salting	<i>Opisthorchis</i> metacercariae in fermented fish	13.6%	24 h	Kruatrachue <i>et al.</i> (1982)
	<i>C. sinensis</i> in naturally infected fish	30% (wt based)	8 d	Fan (1998)
	<i>O. viverrini</i> metacercariae in fermented fish	20% (wt based)	5 h ^a	Tesana, Kaewkes & Phinlaor (1986)
Freezing	<i>C. sinensis</i> in naturally infected fish ²	-12 °C	20 d ^b	Fan (1998)
	<i>C. sinensis</i> in naturally infected fish ²	-20 °C	3-4 d ^c	Fan (1998)
	<i>O. felinus</i> in fish	-28 °C	32 h ^a	Recommendation, Ministry of Health, USSR (1990)
	<i>O. felinus</i> in fish	-40 °C	7 h ^a	Recommendation, Ministry of Health, USSR (1990)
	<i>O. felinus</i> in fish	-28 °C	20 h ^a	Fattakhov (1989)
	<i>O. felinus</i> in fish	-35 °C	8 h ^a	Fattakhov (1989)
	<i>O. felinus</i> in fish	-40 °C	2 h ^a	Fattakhov (1989)

^a Viability was markedly reduced but not completely inhibited.

^b Ten days had no inactivating effect, and 18 days had only marginal inactivating effect.

^c Seven days at -20 °C had no inhibitory effect on 10 rats infected, but 3 days storage at -20 °C, followed by thawing and re-freezing for 4 days had 100% inhibitory effect on 10 infected rats.

Intestinal flukes: Intestinal flukes have many biological and epidemiological traits in common with liver flukes (Table 33). The predominant group of fish-borne flukes is the Heterophyidae family (more than 35 species are reported to be zoonotic). These are often referred to as the “minute flukes” because of their small size (usually less than 2.5 mm long as adults). The Heterophyidae belong to the same superfamily (Opisthorchioidea) to which the liver flukes belong.

The other important trematode group is the echinostomes (e.g. *Echinostomus* and *Echinostoma*) of the Echinostomatidae family, although the number of zoonotic species is much smaller (about ten). These intestinal flukes have a very wide fish host range (at least 45 genera) and share much of this host range with liver flukes. However, they differ from liver flukes in several important respects. Along with many species of fish-eating mammals, intestinal flukes are, for the most part, also infective for fish-eating birds. Moreover, they utilize different snail host species than those used by liver flukes.

Although generally not considered on the same level of significant clinical importance as liver flukes, several heterophyid species, including *Stellantchasmus*, *Metagonimus*, *Haplorchis* and *Procerovum*, can cause significant pathology, infrequently fatal, in the heart, brain and spinal cord of humans (Africa, Leon and Garcia, 1940; WHO, 1995). The exact mechanisms responsible for pathogenesis are not clear but may be related to invasion of the circulatory system by worm eggs. Disease is usually related to worm burdens, which can be very heavy in some cases.

Another important issue related to heterophyids is the difficulty of differentiating their eggs from those of liver flukes in human faecal examinations, which may cause inaccurate estimates of the prevalence of both trematode groups (Chai and Lee, 2002; Ditrich *et al.*, 1992). New diagnostic techniques to improve specific diagnosis of these flukes' faecal eggs are needed. It is more common to encounter multiple-species infections rather than single-species infections, which compounds the problem of

diagnosis by faecal examinations (Lee *et al.*, 1984; Ditrich *et al.*, 1992; Chai *et al.*, 2008; Dung *et al.*, 2007).

Prevalence and infection levels of intestinal fluke metacercariae are often very high in marine/brackish-water and freshwater fish, especially in Asia (WHO, 1995; Chi *et al.*, 2008; Dung *et al.*, 2007; Rim *et al.*, 2008). Therefore, human infections with intestinal flukes are often more frequent than liver flukes, which were previously considered to be more prevalent (Chai, Murrell and Lymbery, 2005; Dung *et al.*, 2007). Because of the ecological and epidemiological similarities between liver and intestinal flukes, their prevention and control approaches are similar (Figure 15; Tables 33 and 34).

Cestodes: Infections with *Diphyllobothrium* spp. (often termed the broad fish tapeworm) are the most important of the cestode parasites acquired by humans from eating improperly cooked or prepared fish (Table 35).

TABLE 35

Distribution, biology, transmission and public health risks for fish-borne cestode infections

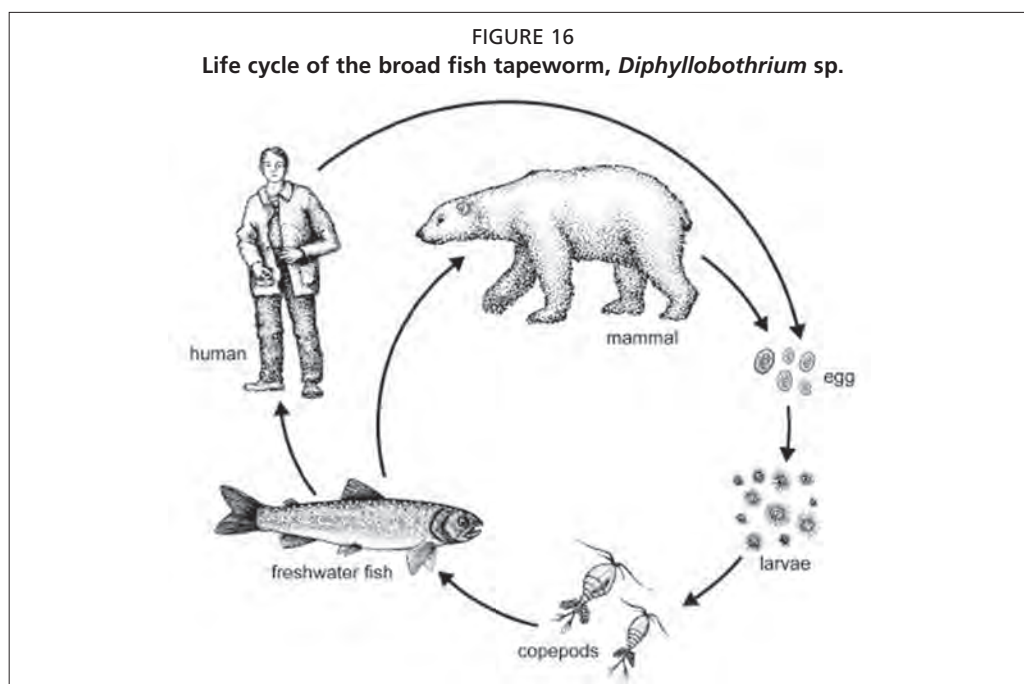
Parasite – Cestodes (tapeworms)	Major geographic distribution	Biology and transmission features	Risk factors
<i>Diphyllobothrium</i> spp. (broad tapeworm).	North and South America, and Eurasia.	Adult worms in intestines of wild fish-eating birds and mammals (especially dogs, bears, fur seals and sea lions), and humans.	Consumption of raw or insufficiently cooked, smoked, dried or pickled fish are the major risk factors.
More than a dozen species have been identified as zoonotic.		Copepods are the major first intermediate host for the first development stage, the proceroid. Ingestion by a fish releases the proceroid, which invades the tissue and develops to the second stage (plerocercoid). Both marine and freshwater fish are important, especially pike, salmon, trout, ruff, white fish, and perch. When uncooked fish is eaten by a mammal or bird host (depending upon species of <i>Diphyllobothrium</i>), the plerocercoid develops to the adult stage in the intestine.	Wild animal reservoirs ensure presence of these helminths in endemic areas, and intrusion of humans into aquatic habitats increases exposure. Insufficient handling of human waste in wilderness areas is a significant risk factor for fish, animals and humans. Importation and stocking of fish may be a significant factor in increasing spread.

Source: Modified from Murrell and Crompton (2009).

The distribution of the tapeworm is widespread in the temperate and sub-Arctic regions of the Northern Hemisphere where freshwater fish are eaten. All are gastrointestinal parasites as adults in a variety of piscivorous birds and mammals. The intermediate hosts include both freshwater and marine fish, especially anadromous species. Although not generally considered a serious zoonosis, there are indications that its frequency and distribution is increasing in some regions, probably because of social and economic change. Although most human infections are diagnosed as *D. latum*, this species is only one of several members of a group (perhaps 13 species) that is a species-complex worldwide zoonosis (Dick, 2007). The systematics of this group is complex, and is currently being unravelled with new molecular methods. The importance of a sound taxonomy and ecological understanding of this group lies in clarifying its epidemiology. Some species, especially *D. latum*, appear to be linked to a cycle that involves mainly freshwater fish and terrestrial mammals, while others, such as *D. nihonkaiense*, may be primarily a parasite of marine fish and mammals. Because these parasites are difficult to differentiate morphologically, the actual species geographic distributions, host ranges and zoonotic risks must be described with some caution (Dick, 2007).

These tapeworms are among the largest parasites of humans, and may, as adults in the intestine, grow to 2–15 m in length. The long chain of segments (stobila) is headed by a scolex with a dorsal–ventral sucking groove (or bothrium) that functions as a holdfast in the intestine. The strobila may contain up to 3 000 segments or proglottids.

Life cycles (Figure 16) are known for only a few of the species, but those that have been described are complex, requiring three hosts for completion, and additional or paratenic hosts may also be involved (Rausch and Adams, 2000).



Source: Huss, Ababouch and Gram (2004).

The zoonosis occurs most frequently in communities that have food preferences for fish prepared in a variety of ways, particularly raw fish preparations, such as sushi and sashimi, which have found worldwide popularity. Others are Scandinavian gravlax, stroganina in the Baltic countries and Eurasia, gefilte fish, and lightly marinated fish dishes such as ceviche salad, which is growing in popularity in Latin America (Adams and Rausch, 1997; Dupouy-Camet and Peduzzi, 2004; Dick, 2007). An increasingly important factor in introducing or sustaining this zoonosis in human communities is the contamination of the local aquatic environment with faeces contaminated with eggs (Cross, 2001; Dupouy-Camet and Peduzzi, 2004). The discharge of improperly treated sewage from lake-side dwellings, hotels and ships is an important source of contamination with eggs. Domestic animals, especially dogs, are another important source of environmental contamination (Adams and Rausch, 1997; Torres *et al.*, 2004), and may help maintain a natural *D. latum* cycle that can be amplified by human activities (Dick, Nelson and Choudhury, 2001).

The growing awareness of the nutritional benefits of fish and fish products, the preferences in many countries for raw or lightly cooked foods, and the rising affluence in both developing and developed countries may increase the risk of diphyllobothriids entering the human food chain by increasing the harvesting and export of fish from areas of high endemicity. Higher risks for urban populations may also arise because of the incentive for exporters to ship fresh (non-frozen) fish by air to gain a competitive edge in the market (Deardorff and Overstreet, 1991; Nawa *et al.*, 2001).

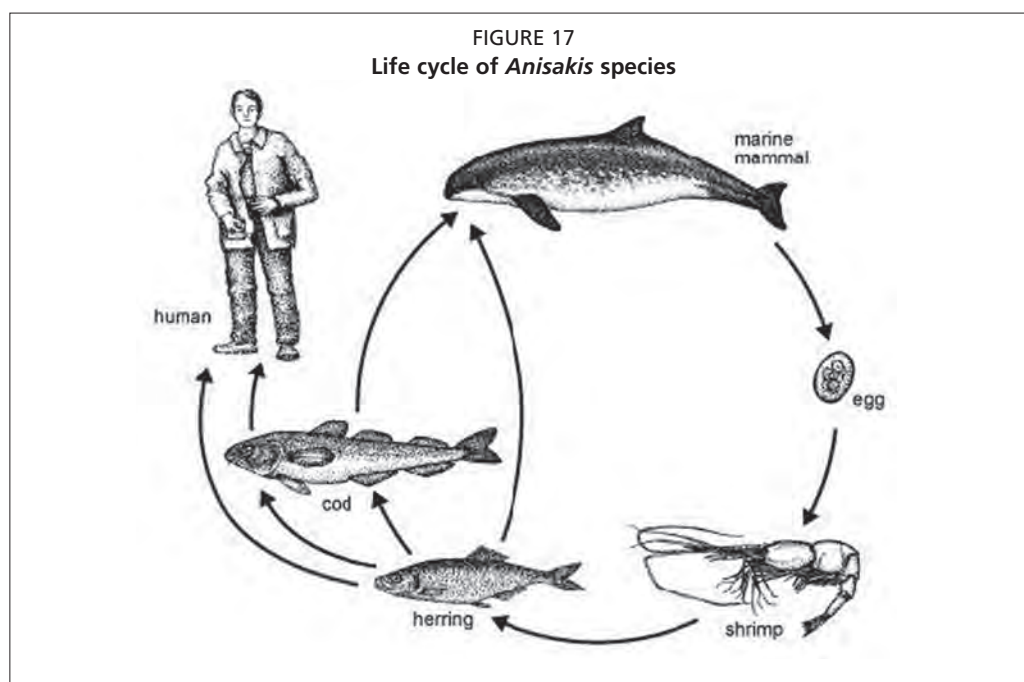
The prevention and control of *Diphyllobothrium* spp. follows a strategy similar to that recommended for trematode zoonoses (Figure 15) – the prevention of human waste from entering untreated into the aquatic system is paramount, because the intermediate and definitive hosts are mainly found wild in nature, making interventions to prevent fish infections impractical. The proper preparation of fish dishes is especially important for consumers (Adams, Murrell and Cross, 1997). The pleurocercoids

(infective stage in fish) can be destroyed by heating to 56 °C for 5–10 min or freezing to –23 °C for 7 days or –35 °C for 15 h. Government inspection of fish fillets is mandated in some instances in a few countries (e.g. Canada, the United States of America and the European Union [Member Organization]), and is accomplished by candling of fillets.

3.2.4.2 Nematodes

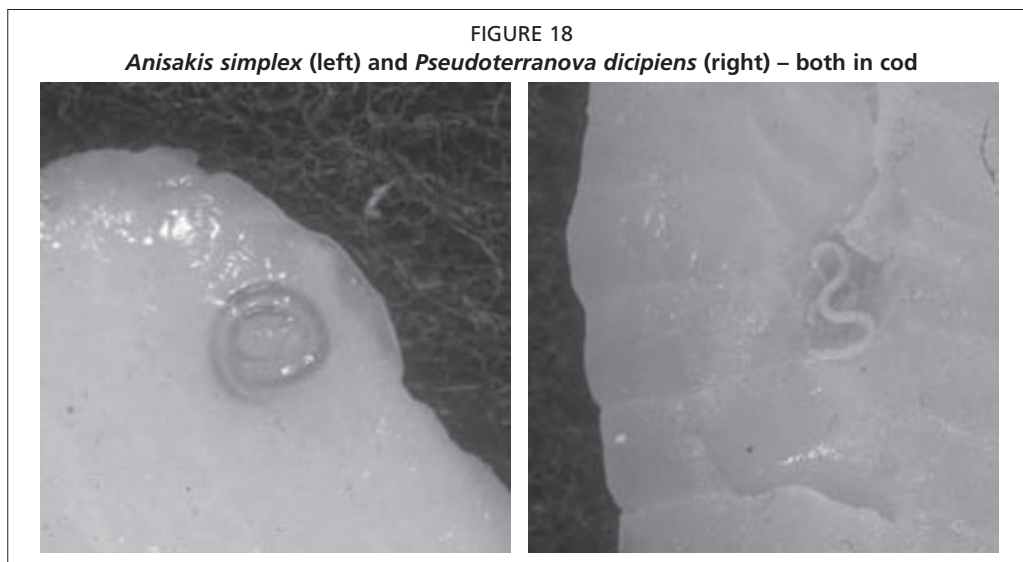
Anisakis: Anisakiasis refers to infection of people with the larval stages of nematodes belonging to the families Anisakidae. Although cases of human infection have been reported with several species (Smith and Wootten, 1987), the two parasites most often associated with anisakiasis are *Anisakis simplex* and *Pseudoterranova decipiens* (Table 36). Species identification in *Anisakis* has long been complicated by a lack of distinguishing morphological characteristics, particularly in the larval stage. Historically, therefore, only two major zoonotic species were recognized: the “herring worm”, *Anisakis simplex*; and the “codworm” *Pseudoterranova* (syns *Phocanema*, *Terranova*) *decipiens*, both with a potentially cosmopolitan distribution (Smith and Wootten, 1987; Oshima, 1987). However, recent molecular genetic studies have shown that both of these morphospecies are actually comprised of a number of sibling species, often with distinct geographical and/or host ranges. At least three species have been described within the *Anisakis simplex* complex: *A. simplex* (*sensu stricto*), found in the northern Atlantic; *A. simplex* C, found in the northern Pacific and southern waters below 30°N; and *A. pegreffi*, found in the Mediterranean Sea (Mattiucci *et al.*, 1997; Lymbery and Cheah, 2007). Three species have now been described for the *Pseudoterranova decipiens* complex: *P. decipiens* A, in the northeast Atlantic and Norwegian Sea; *P. decipiens* C in the northwest Atlantic and Barents Sea; and *P. decipiens* B throughout northern waters. Where the ranges of these species overlap, they appear to preferentially utilize different definitive host species (Lymbery and Cheah, 2007).

Anisakiasis occurs when people ingest third-stage larvae found in the viscera or muscle of a wide range of fish and cephalopod mollusc species. Humans are an accidental host in the life cycle, and the parasites almost never develop further within the human gastrointestinal tract (Figure 17).



Source: Huss, Ababouch and Gram (2004).

Anisakiasis is a serious zoonotic disease, and there has been a dramatic increase in its reported prevalence throughout the world in the last two decades (Lymbery and Cheah, 2007). The complex life history of *A. simplex* involves an intermediate host (euphasid crustacean), a paratenic host (marine fish or squid) and a definitive host (marine mammal). Adult nematodes live in nodules in the stomach linings of cod (Figure 18).



Note: Photographs courtesy of S. Møllgaard.
Source: Huss, Ababouch and Gram (2004).

Eggs are passed into the sea and the embryos develop to release second-stage larvae. These change into third-stage larvae after being eaten by crustacean intermediate hosts. When infected crustaceans are eaten by fish, the third-stage larvae encyst in the fish tissues without further development. Muller (2001) states that 164 species of marine fish can serve as hosts for *Anisakis*. In the natural course of events, predatory marine mammals acquire *Anisakis* by eating infected fish, and the helminth's life cycle is completed (Lymbery and Cheah, 2007). The life cycle of *Pseudoterranova decipiens* is similar. An important aspect of the life cycle of species of both *Anisakis* and *Pseudoterranova*, from an epidemiological perspective, is that larval parasites will readily transfer from one host to another, and piscivorous fish can therefore accumulate very large numbers of larvae (Smith and Wootten, 1987).

When humans, in contrast to marine mammals, eat infected fish harbouring live third-stage larvae, the larvae migrate to the gastrointestinal mucosa, but they do not develop to adult worms; they die and so induce the formation of abscesses (Lymbery and Cheah, 2007). Presumptive diagnosis in humans may be made on the basis of the patient's recent food habits. Definitive diagnosis requires demonstration of worms by gastroscopy or surgery (Markell, John and Krotzski, 1999). No treatment is recommended for transient infection. In the gastrointestinal form (embedded larvae), diagnosis by surgery or gastroscopy is also curative.

Anisakiasis occurs throughout the world, but is reported most frequently from north Asia (especially Japan) and western Europe, where groups have risky food behaviour customs (i.e. eating raw, lightly cooked or marinated fish in dishes such as sushi, salted or smoked herring, gravlax and ceviche). Prevalence in fish may differ considerably between regions and wild and farmed salmon; no infections have been reported from the latter (Table 37). The reasons may be complex, ranging from rearing fish in areas

with no presence of sea mammals (definitive hosts) to increasing populations of marine mammals in other regions (e.g. north Pacific) due to greater regulatory controls (Lymbery and Cheah, 2007). The greater number of human cases reported in recent years may also be related to better diagnostic tools, increased demand for seafood, and a growing demand for raw or lightly cooked food, although none of these factors has been rigorously evaluated (Chai, Murrell and Lymbery, 2005).

TABLE 36
Distribution, biology, transmission and public health risks for fish-borne nematode infections

Parasite – Nematodes	Major geographic distribution	Biology and transmission features	Risk factors
<i>Anisakis simplex</i> <i>Pseudoterranova decipiens</i>	Worldwide but especially important in Northern Europe, Japan, the Republic of Korea, North America, and Pacific Islands.	<p>Complex life cycles involving marine mammals (definitive hosts) such as dolphins, porpoises, and whales (<i>Anisakis</i>) or seals, sea lions and walrus (<i>Pseudoterranova</i>).</p> <p>First intermediate hosts are marine crustaceans, in which early parasite larval development to the third stage occurs.</p> <p>When eaten by a fish or squid (paratenic host), the larvae penetrate the intestine and invade the tissues. The parasites complete their development to adults in the intestines of marine mammals when infected fish or squids are eaten. In these hosts, the larvae develop to adults in the intestine. However, in humans eating raw paratenic hosts (fish, squids), the larvae may remain in the intestine (asymptomatic) or encyst in the stomach wall.</p> <p>Major fish host species are, herring, cod, mackerel, salmon, tuna, whiting, haddock, smelt and plaice.</p>	<p>Most important risk factor, as with flukes and broad tapeworm, is the consumption of raw, insufficiently cooked, salted, pickled or smoked fish or squid. Examples are traditional celebration and wedding dishes such as raw herring, lomi lomi, marinated salmon, sushi, sashimi, ceviche salad, and sunomono.</p>
<i>Gnathostoma</i> spp.	Mainly Southeast Asia and Latin America	<p>The definitive hosts for these parasites are normally carnivorous mammals, including cat, dog and pig. Eggs that are passed out of the definitive host, if reaching water, hatch, releasing larvae that are eaten by copepods, which in turn are eaten by a second intermediate host (fish, amphibians, reptiles, birds and mammals). In these hosts, the larvae develop to the third stage and, when eaten by a potential definitive host, the larvae make a complex extra-intestinal tissue migration, eventually returning to the stomach to form a tumour-like mass in the gastric wall. The worms reach maturity, reproduce, and release eggs that are passed out in the faeces. In people, the worms do not mature, but migrate through the tissues, and are serious if they invade the central nervous system.</p>	<p>As for all food-borne zoonoses, thorough cooking or freezing of all food sources is effective.</p> <p>Because of the diverse sylvatic (wild animal) host range, removing this parasite from the food chain in endemic areas is not possible.</p>

TABLE 37

Prevalence of *Anisakis simplex* in reared and wild-caught marine fish species

Fish	Origin	Number of samples	% positive
Farmed salmon	Washington	50	0
Farmed salmon	Norway	2 832	0
Farmed salmon	Scotland	867	0
Farmed coho salmon	Japan	249	0
Farmed rainbow trout	Japan	40	0
Wild salmon	Washington	237	100
Wild salmon	North Atlantic	62	65
Wild salmon	West Atlantic	334	80–100
Wild salmon	East Atlantic	34	82
Wild coho salmon	Japan	40	100
Sardines	Mediterranean	7	14
Herring	Mediterranean	4 948	86
Herring	Pacific Ocean	127	88
Cod	Pacific Ocean	509	84

Source: ICMSF (2003).

An important factor associated with risk for humans is the commercial methods employed to catch and transport fish. Eviscerating fish shortly after they are caught removes much of the danger that larvae will be able to migrate out of the viscera and into the fish muscle, which is the part of the fish normally consumed. In fish that are caught but then held on ice or under refrigeration for several days, larval migration may be facilitated. However, the extent of post-mortem migration of larvae has not been evaluated thoroughly, although most control measures emphasize immediate evisceration (Lymbery and Cheah, 2007).

While cooking (60 °C or higher for at least 10 min) is effective in killing the larvae in fish, other methods are also capable of inactivating the infective larvae. Many countries have regulations requiring inspection of fish for zoonotic parasites, and for inactivating any nematode larvae that may be present. However, regulations and methods may differ between countries somewhat in their specifics. For example, according to the United States Food and Drug Administration, freezing of fish or cephalopods to –20 °C for 7 days or –35 °C for 15 h is considered sufficient to render them safe enough to be eaten raw (FDA, 2011e). Smoking must achieve a temperature of 65 °C. Salting and marinating are not considered reliable methods to inactivate the larvae. In the European Union (Member Organization), conditions laid down in Council Directive 91/493/EEC and Commission Decision 93/140 stipulate that all fish and fish products to be consumed raw or almost raw must be subjected to freezing to –20 °C for at least 24 h in all parts of the fish (EC, 1991). Fish products that are heated (e.g. hot-smoked) to a temperature of less than 60 °C must also have been first subjected to freezing by the same standards.

A number of well-known fish products can be unsafe. This applies to all lightly preserved fish products (< 5 percent NaCl in water phase) such as cold-smoked fish, gravad fish, matjes herring, lightly salted caviar, ceviche and several other local traditional products. Both the European Union (Member Organization) and FDA regulations include inspection of fillets by candling.

Gnathostoma: Gnathostomiasis is a zoonotic disease caused by species of the nematode *Gnathostoma*, which can be transmitted by a variety of intermediate hosts, including freshwater fish (Table 36) (Waikagul and Chamacho-Diaz, 2007). Human infections with this nematode are frequently reported in Southeast Asia and Latin America. The infection is characterized as a type of “larval migrans”, in which larvae may invade not only subcutaneous tissue but, more seriously, the central nervous system and the eye.

These zoonotic nematodes are composed of numerous species, five to ten of which are associated with human infection (Waikagul and Chamacho-Diaz, 2007). *Gnathostoma spinigerum* is the most commonly reported species in humans. The definitive hosts for these parasites are normally carnivorous mammals, including cat, dog and pig. An important morphological feature of this parasite is its subglobulus head, armed with 7–9 transverse rows of hooklets, which probably facilitates larval tissue migration, and, consequently, contributes to the tissue damage that occurs in the host's organs and tissues. The life cycle is complex and involves a wide range of intermediate hosts (Waikagul and Chamacho-Diaz, 2007). Eggs that are passed out of the definitive host hatch on reaching water, releasing larvae that are eaten by copepods, which in turn are eaten by a second intermediate host (fish, amphibians, reptiles, birds and mammals). In these hosts, the larvae develop to the third stage. When eaten by a potential definitive host, the larvae make a complex extra-intestinal tissue migration, eventually returning to the stomach to form a tumour-like mass in the gastric wall. The worms reach maturity, reproduce, and release eggs that are passed out in the faeces.

Although prevalence data are few, this zoonosis has been reported extensively throughout Southeast Asia, where the fondness for raw or undercooked intermediate hosts such as fish, frogs, snakes and poultry is strong (Waikagul and Chamacho-Diaz, 2007). In recent years, cases of gnathostomiasis have been increasing in Argentina, Peru, Ecuador and Mexico, where it is now recognized as an important public health risk (Waikagul and Chamacho-Diaz, 2007). As for all food-borne zoonoses, the thorough cooking or freezing of all fish (and other risky foods) is effective for inactivating the infective larvae (see *Anisakis* above). Because of the diverse wild animal host range, removing this parasite from the food chain in endemic areas is not possible. However, while systematic prevalence surveys are few, in Viet Nam, wild but not cultured eels have been found infected (Sieu *et al.*, 2009), a situation similar to that for *Anisakis*.

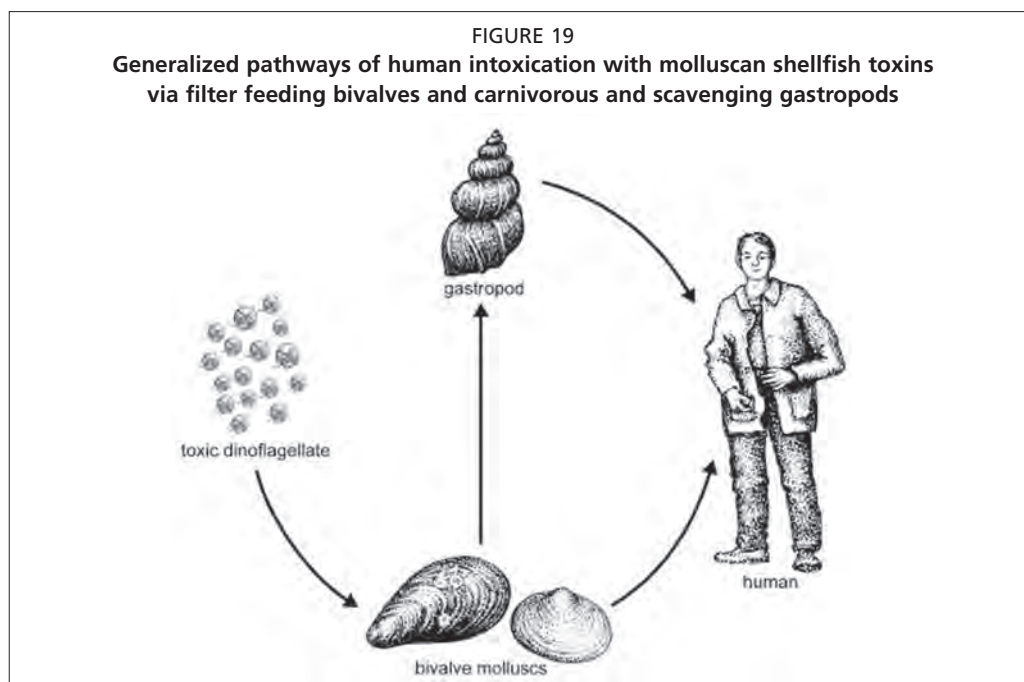
Conclusion: The growing awareness of the nutritional benefits of fish and fish products, the preferences in many countries for raw or lightly cooked foods, and the rising affluence in both developing and developed countries may increase the risk of fish-borne parasites entering the human food chain by increasing the harvesting, transport and export of fish from areas of high endemicity. Higher risks for urban populations may also arise because of the incentive for exporters to ship fresh (non-frozen) fish by air to gain a competitive edge in the market. Urbanization and insufficient sanitary conditions (e.g. discharge of improperly treated sewage) as well as heavy rain may lead to increased faecal contamination of the aquatic environment. At the same time, increased aquaculture production is projected worldwide in such aquatic environments. An integrated HACCP-based approach including measures to prevent and control pollution with animal- and human-parasite eggs, control of animal and snail intermediate hosts, and better aquaculture management practices are needed for sustainable control of fish-borne zoonotic parasites.

3.2.5 Aquatic biotoxins (Jim Lawrence, Henri Loreal and Lahsen Ababouch)

The possible presence of natural toxins in fish and shellfish has been known for a long time. Most of these toxins are produced by species of naturally occurring marine algae (phytoplankton). There are about 5 000 species of marine algae, but only 70–80 species are known to produce toxins (Lindahl, 1998; Hallegraeff, McCausland and Brown, 1995).

A proportion of the toxic phytoplankton has a red-brown pigmentation, giving rise to the naming of algal blooms as “red tides”. However, not all coloured algae are toxic, and incidences of poisoning have occurred in the absence of red tides. Visible red tides may contain from 20 000 to greater than 50 000 algal cells per millilitre. Concentrations as low as 200 cells/ml may lead to toxic shellfish. During a bloom,

bivalves can accumulate sufficient toxin to cause human illness after filter feeding for only 24 h (Figure 19). Most harmful algal species have limited geographic distributions but some occur worldwide (Hallegraeff, McCausland and Brown, 1995; Lindahl, 1998).



Source: From Anderson et al. (2001).

In the past 25 years, the intensity and geographical distribution of harmful algal blooms have increased. In addition, the number of toxic substances produced by marine algae appears to be increasing. There are several possible causes of this. There is evidence from Europe and Asia that eutrophication from domestic, industrial and agricultural runoff can stimulate algal blooms (Anderson, Glibert and Burkholder, 2002). Increased shipping trade and the practice of dumping ballast water contributes to the global spread of algal blooms (Wright, 1995). Global warming may be implicated as well.

Molluscan shellfish are filter feeders and continually pump water through their gills, where particulate matter is removed and ingested. Mussels ingest food of any type from 2 mm to 90 mm in size with a rate of ingestion dependent on water temperature and environment. Optimally, they can filter 2.5 litres per hour, extracting 98 percent of the available algae. Consequently, any toxins associated with the phytoplankton can accumulate and become concentrated in the bivalve mollusc. The toxins do not affect the shellfish themselves, and the shellfish may reduce the concentration of the toxins in their system by depuration in clean water. Depuration times vary greatly according to bivalve species, the pumping activity and the hydrographic conditions. Fish may also consume toxic algae and cause human illness (e.g. ciguatera). There are also toxins in some fish species that do not involve marine algae (e.g. puffer fish poisoning). The consumption of toxic fish and shellfish by humans causes illness with symptoms ranging from mild diarrhoea and vomiting to memory loss, paralysis and death.

The toxins associated with phytoplankton are called phycotoxins. These toxins have been responsible for incidents of wide-scale death of sea-life and are increasingly responsible for human intoxication. There are a number of different seafood poisoning syndromes associated with toxic marine algae. They include: paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhoeic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP) and azaspiracid shellfish

poisoning (AZP). Table 38 lists the syndromes, causative agents and the occurrence of these biotoxin-related poisonings. Table 39 lists typical concentration ranges of several groups of toxins that may lead to closures of shellfish harvesting areas along with maximum reported levels in shellfish and some current regulatory guidelines implemented in some countries. (FAO, 2005a). There are also different types of food poisoning associated with finfish and these include ciguatera fish poisoning (CFP) and puffer fish poisoning (PFP).

TABLE 38

Marine biotoxins and associated poisonings

Disease	Toxins	Occurrence
Amnesic shellfish poisoning (ASP)	Domoic acid	North America, Europe
Azaspiracid shellfish poisoning (AZP)	Azaspiracid	Europe
Diarrhoeic shellfish poisoning (DSP)	Okadaic acid and dinophysistoxins	Worldwide
Neurotoxic shellfish poisoning (NSP)	Brevetoxins	United States of America, Caribbean, New Zealand
Paralytic shellfish poisoning (PSP)	Saxitoxins	Worldwide
Ciguatera fish poisoning (CFP)	Ciguatoxins	Tropical, subtropical
Puffer fish poisoning (PFP)	Tetrodotoxins	Japan, South Pacific

TABLE 39

Typical concentration ranges of several groups of toxins that may lead to closures of shellfish harvesting areas, along with maximum reported levels in shellfish and some current guideline levels

Toxin Group	Typical level when toxins occur at levels that may lead to closure of the area (mg/kg)	Maximum reported level	Guidance level / maximum level currently implemented in some countries
Amnesic shellfish poisoning (ASP)	20–200	1 280	20 mg/kg
Azaspiracid shellfish poisoning (AZP)	0.16–0.3	1.4	0.16 mg/kg
Diarrhoeic shellfish poisoning (DSP)	0.16–1	36	0.16 mg/kg
Neurotoxic shellfish poisoning (NSP)	0.8	40	20 MU/100 g shellfish meat
Puffer fish poisoning (PFP)	1–10	800	0.8 mg/kg

Source: FAO (2005a).

Most algal toxins associated with seafood poisoning are heat stable and are not inactivated or destroyed by cooking. It is also not possible to visually distinguish toxic from non-toxic fish and shellfish. In spite of this, efforts have been made to detoxify contaminated shellfish. Several procedures to detoxify shellfish have been developed to mitigate the economic impact of toxin contamination. For example, the concentration of toxins in the digestive gland of scallops has enabled the harvesting of the non-toxic edible portion for consumption. Concentrations of some water-soluble toxins are reduced through leaching out with the cooking water. Canning has been shown to reduce PSP and ASP toxin concentrations in a number of shellfish species. In these cases, the final products must still be tested to ensure that the toxin concentrations are indeed reduced to acceptable levels.

Many countries rely on biotoxin monitoring programmes to protect public health. Harvesting areas are usually closed when toxic algal blooms or toxic shellfish are detected. In non-industrialized countries, particularly in rural areas, monitoring for harmful algal blooms or toxic shellfish is not routinely carried out, and illness or death from algal toxin poisoning regularly occurs. Historically, the most common testing methods involved animal bioassays, usually with mice or rats. However, these methods are not fully satisfactory as they are prone to interference and lack the ability to

quantify the toxins, making the methods problematic for enforcement purposes. Thus, in recent years, there has been a concerted effort to develop and implement non-animal assays such as *in vitro* assays, immunoassays and instrumental methods. Development of alternative methods has become even more important as a number of countries are actively considering banning the mouse bioassay owing to concerns related to the ethical treatment of animals (Hess *et al.*, 2006). However, the newer techniques require purified analytical standards and reference materials to calibrate instruments and to ensure accuracy of methods for regulatory testing. As there is a great lack of such materials at present, this situation will clearly have an impact on the acceptance and use of non-animal testing methods. This issue has recently been addressed in Hess, McCarron and Quilliam (2007).

3.2.5.1 Amnesic shellfish poisoning

Amnesic shellfish poisoning (ASP) is the only shellfish poison produced by a diatom (*Pseudo-nitzschia pungens* f. *multiseries*). The causative substance was identified as domoic acid, a member of the kainoid class of compounds, which are potent neurotoxins. The illness was first reported in Canada in 1987, where more than 100 people became ill and 3 people died after consuming contaminated shellfish (Todd, 1993). The illness was named after one of the symptoms, which was loss of short-term memory. Other symptoms include nausea, vomiting, diarrhoea, headache and neurological effects such as dizziness, disorientation and confusion. In severe cases, seizures followed by coma and death may occur. The short-term memory loss appeared to be permanent in some survivors. Outbreaks of human poisonings have so far been confined to the initial episode in Canada. However, the presence of domoic acid in shellfish has been reported in the United States of America and many other geographic areas of the world. Because of the potential for human illness, global awareness has now been raised (Hallegraeff, McCausland and Brown, 1995).

A number of testing methods have been developed to detect domoic acid in shellfish. These include *in vitro* functional assays, immunochemical methods and instrumental methods. Although a mouse bioassay was employed in the initial discovery of the syndrome and as an aid to isolating and identifying the toxin, the method is not sensitive enough to be used for regulatory monitoring of shellfish. Thus, high-performance liquid chromatography (HPLC) has been used most often for this purpose. Two HPLC methods have been validated through interlaboratory study, and variations of these are commonly used to monitor shellfish. An enzyme-linked immunosorbent assay (ELISA) method has recently successfully undergone an Association of Official Analytical Chemists (AOAC) International interlaboratory study and is suitable for domoic acid testing shellfish in a routine setting.

3.2.5.2 Azaspiracid poisoning

Azaspiracid poisoning (AZP) is a recent syndrome first reported in 1995 when eight people in the Netherlands became ill from eating cultured mussels imported from Ireland. The symptoms were similar to DSP and included nausea, vomiting, stomach cramps and severe diarrhoea. The causative agent was subsequently identified as a group of chemicals called azaspiracids (AZAs), of which 10 analogues have since been identified. They have unique spiro ring assemblies and contain a cyclic amine group. Since the initial finding, AZAs have been found in other countries in Europe, and the European Union (Member Organization) has set a regulatory limit for this class of toxin at 0.16 µg/g total shellfish tissue. The source of AZAs has been confirmed to be a dinoflagellate of the genus *Protoperidinium*. Like other polyether toxins, AZA compounds are heat stable and not affected by cooking. The toxins are also not readily removed from shellfish by natural depuration. (FAO, 2004, 2005a).

The current reference method in the European Union (Member Organization) is a mouse bioassay, although the method has not been validated in terms of detectability and specificity. Instrumental methods using HPLC with mass spectrometric detection have shown considerable potential for quantifying AZAs in shellfish tissue. One such method (a multitoxin method) has passed single-laboratory validation and limited interlaboratory validation (McNabb, Selwood and Holland, 2005).

3.2.5.3 *Diarrhoeic shellfish poisoning*

Toxins in the diarrhoeic shellfish poisoning (DSP) group have been known to cause human illness since the late 1970s. The syndrome was named diarrhoeic shellfish poisoning in view of the dominating symptom. The toxins are produced by dinoflagellates of the genera *Dinophysis* spp. and *Prorocentrum* spp. The symptoms are gastrointestinal (diarrhoea, vomiting and abdominal pain) and victims usually recover in 3–4 days with or without treatment. No fatalities have ever been observed (FAO, 2004, 2005a).

Thousands of cases of gastrointestinal disorders caused by DSP have been reported in Europe, Japan, Southeast Asia, and North and South America since the 1970s. The causative dinoflagellates (*Dinophysis* and *Prorocentrum*) are widespread in the oceans, meaning that DSP could potentially occur in many other parts of the world. The major toxins that have been identified as causing the illness are members of the okadaic acid group. These toxins are heat-stable, lipophilic polyether compounds. The DSP group previously consisted of the acidic okadaic acid family (including okadaic acid, dinophysistoxins [DTX-1 and DTX-2] and their esters), the pectenotoxins (PTXs) (neutral group) and the sulphated group, the yessotoxins (YTXs). The three groups were all considered as DSP toxins because they regularly occur together in toxic algal blooms and shellfish. However, recent toxicology studies have indicated that the PTX and YTX groups do not cause diarrhoea when fed via the oral route. There have been no human illnesses reported due solely to the PTX and YTX groups. However, these groups are toxic to mice when injected interperitoneally and, thus, can cause interference in the detection of the okadaic acid group by this bioassay. It is thus important that testing methods be able to distinguish among these groups. Because the DSP toxins are heat stable, they are not destroyed by normal cooking (FAO, 2004, 2005a).

Several mouse bioassays have been developed for detecting DSP toxins. However, they suffer from potential interference from the PTX, YTX and cyclic imine compounds. Alternative approaches such as *in vitro* functional assays, immunochemical assays and instrumental methods have been developed. None has undergone independent multilaboratory validation. However, an HPLC mass spectrometric method (McNabb, Selwood and Holland, 2005) has undergone full single-laboratory validation and limited interlaboratory testing. At present, HPLC mass spectrometry offers the best potential for quantifying DSP toxins in shellfish.

3.2.5.4 *Neurotoxic shellfish poisoning*

Neurotoxic shellfish poisoning (NSP) is caused by a group of polyether toxins called brevetoxins. They are produced by the dinoflagellate, *Karenia brevis*. The occurrence of NSP has been historically limited to the west coast of Florida, the United States of America. The dinoflagellate occurs offshore in the Gulf of Mexico and is carried in-shore by winds and currents. There have also been NSP outbreaks in New Zealand (FAO, 2004, 2005a). The symptoms of NSP are similar to PSP and ciguatera poisoning, but less severe. They occur within from 30 min to 3 h after ingestion and last a few days. They include nausea, vomiting, diarrhoea, chills, sweating, hypotension, arrhythmias, tingling, numbness, paralysis, seizures and coma. No deaths have been reported. The toxins are toxic to fish and have caused significant fish kills. Detoxifying contaminated

shellfish is usually done by natural depuration in clean water. Cooking and freezing are ineffective in destroying the toxins.

About ten brevetoxins have been isolated from field blooms and cultures of *K. brevis*. Additional related toxins have been isolated from shellfish. The most commonly used testing method has been a mouse bioassay that has been used to monitor shellfish effectively in the southeast of the United States of America for more than 30 years. It has not been fully validated, and there are some concerns regarding the extraction efficiency and quantitative aspects. *In vitro* assays, immunoassays and instrumental methods have been examined for detecting and quantifying brevetoxins in shellfish. None of these has yet been validated through interlaboratory study. However, several have potential to be implemented in routine monitoring programmes.

3.2.5.5 Paralytic shellfish poisoning

Paralytic shellfish poisoning (PSP) in humans is caused by the consumption of shellfish containing PSP toxins. These toxins accumulate in shellfish during grazing on toxin-producing algae including dinoflagellates of the genera *Alexandrium*, *Gymnodium* and *Pyrodinium*. Symptoms of PSP initially involve numbness and a burning or tingling sensation of the lips and tongue that spread to the face and fingertips. These symptoms usually appear within the first 30 min after consumption. This then leads to a general lack of muscle coordination in the arms, legs and neck. Severe cases of PSP have resulted in respiratory paralysis and death, usually within 2–24 h after consumption of the contaminated food. There are an estimated 1 600 cases annually of PSP worldwide, approximately 300 of which are fatal (FAO, 2004). There is a large variation in sensitivity to PSP toxins. Intoxications have followed after oral intake of from 144 µg to 1 660 µg per person, while fatalities have been reported at levels from 300 µg to 12 400 µg per person (van Egmond *et al.*, 1993).

Blooms of toxic algae and outbreaks of PSP occur regularly throughout the world (Figure 20). Shellfish that have fed on toxic dinoflagellates retain the toxins for varying periods depending on the shellfish. Some clear the toxins very quickly and are only toxic during the actual bloom. Others may retain the toxins for a long time, even years. Toxic blooms have become more prevalent in recent years, leading to speculation that coastal pollution and shipping practices have contributed to this. Water temperature must be 5–8 °C for blooms to occur. When the temperature decreases to less than 4 °C, the dinoflagellates survive as cysts buried in the upper layer of the sediments.

The toxins associated with PSP belong to a family of water-soluble, polar and heat-stable compounds consisting of a tetrahydropurine nucleus (commonly called saxitoxins). There are four subgroups including carbamate, N-sulpho-carbamoyl, de-carbamoyl and deoxydecarbamoyl. Approximately 21 toxins in this family have been chemically identified. The N-sulpho-carbamoyl analogues are the least toxic of the PSP group (about 5–100 times less toxic, depending upon the specific analogue). Cooking contaminated shellfish for 5 min has been shown to reduce toxin concentrations by about 30 percent. Cooking for 20 min leads to a 40 percent reduction in toxin concentration (FAO, 2004).

Until recently, the mouse bioassay was the standard analytical method employed by regulatory agencies around the world for routine monitoring for the presence of PSP toxins in shellfish. However, in the past decade or more, much research effort has been expended to develop alternative testing methods. This is because of some inherent problems associated with the mouse bioassay (poor detection limit, interference and interconversion of non-toxic analogues to toxic ones during the extraction). Alternative methods include *in vitro* functional assays, immunoassays and instrumental methods (FAO, 2005a). However, most of these have not been validated through interlaboratory collaborative study. Only one method, an HPLC method (Lawrence, Niedzwiadek and Menard, 2005) has met with official approval from

AOAC International for shellfish testing. It is currently in use in the United Kingdom of Great Britain and Northern Ireland as a replacement for the mouse bioassay for regulatory testing of mussels (Cefas, 2011).

FIGURE 20
World distribution of outbreaks of paralytic shellfish poisoning (PSP) in 2006



Source: WHOI. 2103. Distribution of HABs throughout the World. In: *Harmful algae* [online]. [Cited 4 July 2013]. www.whoi.edu/redtide/regions/world-distribution

3.2.5.6 *Ciguatera fish poisoning*

Ciguatera fish poisoning (CFP) is one of the most common food-borne illnesses related to finfish consumption. It has been known for centuries. Its true incidence is not known, but it is estimated that 10 000–50 000 people per year suffer from this illness, making it one of the most common types of marine food-borne poisoning worldwide. It is caused by the consumption of herbivorous fish that have become toxic from feeding on toxic benthic dinoflagellates (*Gambierdinus toxicus*) or from carnivorous fish that have consumed toxic herbivorous fish that have fed on the dinoflagellate. *Gambierdinus toxicus* is found primarily in the tropics in association with macro algae usually attached to dead corals. More than 400 species of fish are known to be vectors of ciguatera poison. These fish are usually found in the tropical and subtropical Pacific and Indian Ocean regions and the tropical Caribbean. Although in the past CFP was highly localized to coastal communities in tropical regions, with the great increase in international trade in seafood and with tourism now on a global scale, the occurrence of CFP has become international.

The chemicals responsible for CFP are called ciguatoxins and arise from the biotransformation in fish of precursor toxins produced by the dinoflagellates. The toxins are lipophilic polyether compounds consisting of 13–14 rings fused together by ether linkages into a rigid ladder-like structure. More than 20 ciguatoxin analogues have been isolated and identified. Like most marine toxins, they are heat stable and remain toxic after cooking. Mild acid and mild basic conditions have little effect on their stability. Depuration is also not effective as contaminated fish tissue can remain toxic for years.

Ciguatoxins, when present at concentrations of about 0.1 µg/kg or greater in fish will cause human poisoning. The symptoms vary widely but are characterized by gastrointestinal, neurological and cardiovascular disturbances often within 10 min and up to 24 h after ingestion of toxic fish. The initial symptoms are gastrointestinal and are similar to other types of food poisoning (abdominal pain, nausea, vomiting and diarrhoea), while the neurological symptoms include tingling and numbness in the mouth, hands and feet, muscle cramping, headache, vertigo and convulsions. Cardiovascular effects such as slow irregular pulse and low arterial pressure may follow and last for 48–72 h. Neurological effects may last for weeks, even years in severe cases. Death from CFP is rare (less than 1 percent worldwide).

The most commonly used testing method for ciguatoxins is the mouse bioassay (FAO, 2004). However, like most other mouse bioassays for marine toxins, the method is not fully quantitative and suffers from interference and ethical issues. *In vitro* functional assays involving sodium channel binding are more sensitive than the mouse assay and have good potential as replacements. Immunoassays have been developed for ciguatoxin detection and do have potential for routine monitoring of fish, although differences in cross-reactivity among the many congeners might affect quantification. Instrumental methods involving HPLC with ultraviolet (UV), fluorometric or mass spectrometric detection have been developed. However, none of these has been validated for the quantification of ciguatoxins for regulatory purposes (FAO, 2005a).

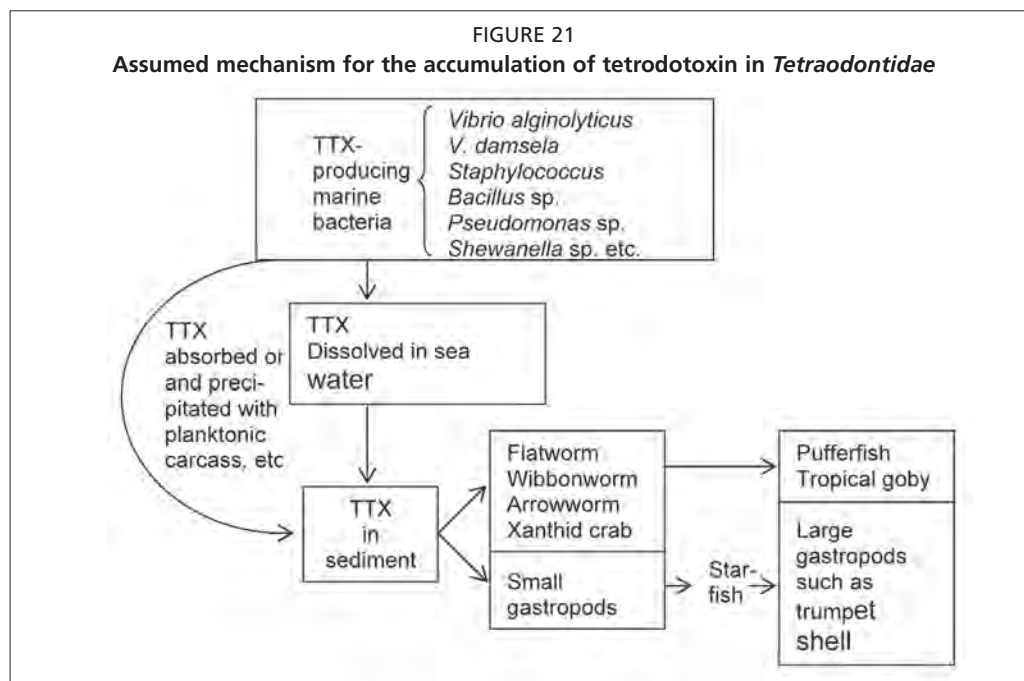
3.2.5.7 Puffer fish poisoning

Puffer fish poisoning (PFP) is an illness specifically related to the consumption of fish of the order *Tetraodontidae*. The toxin responsible for the syndrome is called tetrodotoxin, a non-proteinaceous, highly toxic neurotoxin. There have been about ten additional related compounds that have been isolated in recent years (Pires *et al.*, 2005). Apart from *Tetraodontidae*, the toxin has been found in goby, blue ringed octopus, various gastropods, newts and horseshoe crabs.

Puffer fish poisoning has frequently occurred in Japan, where these fish are a traditional food. Almost 300 cases were recorded in the 10 year period 1987–1996, with an average mortality rate of 6.6 percent (Noguchi and Arakawa, 2008). Sporadic cases of PFP have been observed in other Asian and Pacific countries. Symptoms of PFP occur within 10 min and rarely more than 6 h after ingestion of toxic fish. Nausea and vomiting may or may not occur. The most common symptoms are tingling or a pricking sensation as well as dizziness. The illness may progress to muscle and respiratory paralysis. Where death occurs, it is usually within 6 h and sometimes as rapidly as 20 min following ingestion. Persons who have not died within 24 h usually recover completely.

The distribution of the toxin in fish is mainly in the ovaries (eggs), liver and skin. The muscle tissue is normally free of toxin. The origin of the toxin has historically been much debated. It is now assumed that tetrodotoxin in fish comes directly from its feed. The toxin is produced by bacteria, adsorbed on or precipitated with plankton then transmitted to animals such as gastropods, starfish, flatworms, etc. and further transmitted to fish and large gastropods. Species such as the *Tetraodontidae* accumulate the toxin while other species do not (Noguchi and Arakawa, 2008). Figure 21 shows an assumed mechanism for the accumulation of tetrodotoxin in *Tetraodontidae*.

Tetrodotoxin is a potent sodium channel inhibitor and, as a result, the mouse bioassay is suitable for its determination. In recent years, a number of *in vitro* assays, immunoassays and instrumental methods (HPLC with fluorescence or mass spectrometric detection) have been developed. However, there are no interlaboratory-validated methods for tetrodotoxin.



Source: After Yoshikawa-Ebesu, Hokama and Nogushi (2001).

3.2.5.8 Other marine biotoxins

All of the above-mentioned biotoxins have been associated with human poisonings, and all cause varying toxic effects in animal studies. However, new bioactive marine chemicals continue to be discovered that have been shown to produce toxic effects in animals. Although human illness due to their presence in seafood has not been reported, it is important to be aware of their presence in the marine environment. With present-day changing cultural and trade practices and the current changing global climatic conditions, these substances might be of concern in the future. One example is the cyclic amine group of chemicals. These include gymnodimine, spirolides, pinnatoxins, prorocentrolide and spirocentrimine. These compounds are acutely toxic to mice when administered by intraperitoneal injection, although the toxicity appears to be significantly less via the oral route. The presence of these toxins in shellfish has been confirmed in North America, parts of Europe, New Zealand and Tunisia (Lawrence *et al.*, 2011).

Another group of toxins known to have caused animal poisonings and human illness are the freshwater cyanobacterial toxins, the microcystins. They are known hepatotoxins and are currently of concern in freshwater fisheries and in drinking-water supplies. Although they are not of major concern in the marine environment, their presence has been reported in brackish-water coastal environments. Thus in the future, they could present a health threat in certain marine areas. Another toxic substance, beta-N-methylamino-L-alanine, a potent neurotoxin that may cause motor neuron disease in genetically susceptible people, has been found to be produced by marine cyanobacteria. The toxin has been found in brackish waters on the east coast of the United Kingdom of Great Britain and Northern Ireland and has been found in concentrated seawater in Hawaii, the United States of America (Banack *et al.*, 2007; Metcalf *et al.*, 2008).

3.3 CHEMICAL HAZARDS

3.3.1 Veterinary drugs (Iddya Karunasagar)

The importance of antimicrobial agents in the protection of animal health has been widely acknowledged, but the negative impacts of the use of these agents in animals raised for food have been a cause of concern. While the issue of selection and spread of antibiotic-resistant bacteria in aquaculture has been deliberated upon for some time, the issue of antimicrobial residues in aquaculture products came to the fore in 2001, following marked improvements in laboratory methods to detect residues. This was followed by disruptions of trade in aquaculture products. The use of antimicrobials in agriculture, animal husbandry and aquaculture in many developing countries is often unregulated and there are very few data on their usage. The World Organisation for Animal Health (OIE) prepared a list of antimicrobials of veterinary importance based on a questionnaire survey of member countries (Table 40). The groups of antimicrobials have been categorized as “veterinary critically important antimicrobials (VCIA)”, “veterinary highly important antimicrobials (VHIA)” and “veterinary important antimicrobials (VIA)”. This categorization was based on two criteria: (i) more than 50 percent of respondents identified the importance of the antimicrobial; and (ii) compounds within the class were identified as essential against specific infections and there was a lack of sufficient therapeutic alternatives. Critically important antimicrobials met both criteria, highly important antimicrobials met either of the criteria, and important antimicrobials met neither criterion. For fish, aminoglycosides, fosfomycin, macrolides, aminopenicillins, carboxypenicillins, phenicols (florphenicol, thiamphenicol), quinolones, sulphonamides and tetracyclines were listed as VCIA, lincosamides as VHIA, and bicyclomycin and novobiocin as VIA. There are a limited number of antimicrobials approved for use in aquaculture in the European Union (Member Organization) and the United States of America (Table 40). Licensing is generally for a specific disease in a specific fish species, e.g. florfenicol for control of furunculosis in salmonids cultured in freshwater, or bicozamycin for treatment of pseudotuberculosis in Perciformes (yellowtail, seabass, seabream, tilapia). Maximum residue levels (MRLs) are prescribed for licensed drugs (e.g. Regulation No. 37/2010 of the European Union [Member Organization]), and withdrawal periods are established.

There is very little information on the quantity of antimicrobials used in aquaculture. In the United States aquaculture industry (catfish, salmon and trout), the usage has been estimated to be 92 500–196 400 kg annually (Benbrook, 2002), and this is about 2 percent of non-medical use in meat and companion animals. In the United Kingdom of Great Britain and Northern Ireland, 2 tonnes of antimicrobials (mainly tetracyclines and potentiated sulphonamides) were used in aquaculture (salmon and trout) in 2000 (Furones and Rodgers, 2009). In Chile, 385 600 kg of antibiotics were used in 2007, while in Canada, 21 330 kg antibiotics were used in 2007 (BurrIDGE *et al.*, 2010). For production of the same aquaculture species, different quantities of antibiotics may be used. For example, in Chile in 2007 and 2008, 385.6 tonnes and 325.6 tonnes of antibiotics were used to produce 300 000 and 400 000 tonnes of salmon, but in the same period in Norway, less than one tonne of antibiotic was used to produce 820 000 tonnes salmon (BurrIDGE *et al.*, 2010). There are no reliable data on antibiotic usage in aquaculture in Asia, which accounts for nearly 90 percent of world aquaculture production (FAO, 2009a).

TABLE 40
Antimicrobial use in aquaculture

Antimicrobials appearing in OIE list ¹	Antimicrobials approved by the United States FDA ²	Antimicrobials approved in the European Union (Member Organization) ³
Spectinomycin	Oxytetracycline	Amoxycillin
Streptomycin	Florfenicol	Florfenicol
Kanamycin	Sulphadimethoxine/ormetoprim	Oxolonic acid
Bicozamycin		Oxytetracycline
Fosfomycin		Flumequine
Lincomycin		Sarafloxacin
Erythromycin		Sulphadiazine + trimethoprim
Josamycin		
Spiramycin		
Novobiocin		
Amoxycillin		
Ampicillin		
Tobicillin		
Florphenicol		
Thiamphenicol		
Flumequin		
Miloxacin		
Oxalonic acid		
Enrofloxacin		
Sulphadimethoxine		
Sulphafurazole		
Sulphamethoxine		
Sulphamonomethoxine		
Trimethoprim + sulphonamide		
Doxycycline, oxytetracycline, tetracycline		

¹ OIE. 2013. *OIE list of antimicrobials of veterinary importance* [online]. Paris. [Cited 4 July 2013]. http://web.oie.int/download/Antimicrobials/OIE_list_antimicrobials.pdf

² FDA. 2013. Animal & Veterinary. In: *FDA* [online]. Silver Spring, USA. [Cited 4 July 2013]. www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/ucm132954.htm

³ Furones and Rodgers (2009).

At the international level, the responsibility of providing advice on risk management concerning veterinary drug residues lies with the Codex Alimentarius Commission (CAC) and its subsidiary body, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The primary responsibility for risk assessment lies with the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The CCRVDF determines the priorities for consideration of residues of veterinary drugs, and JECFA provides independent scientific advice by evaluating the available data on veterinary drugs prioritized by the CCRVDF. The Risk Assessment Policy for Setting of MRLs in Food established by the CAC defines the responsibilities of the CCRVDF and JECFA and their interactions. For establishment of a priority list, the CCRVDF identifies, with the assistance of Members, the veterinary drugs that may pose a consumer safety problem and/or have potential adverse impact on international trade.

JECFA uses a risk assessment process to establish acceptable daily intakes (ADIs) and maximum residue limits (MRLs). Veterinary drugs that are toxic or have carcinogenic potential are not evaluated by JECFA and, therefore, no ADI or MRL is established. Chloramphenicol and nitrofurans, compounds that caused disruptions in trade in aquaculture products, belong to this category, and they are banned for use in food-producing animals in most countries. Currently, there is a Codex MRL only for chlortetracycline/oxytetracycline/tetracycline in fish and shrimp (CAC, 2009b).

However, there are national/regional MRLs for several other antimicrobial agents. In the European Union (Member Organization), the Commission Regulation 37/2010 establishes MRLs for veterinary drugs in foods of animal origin, including aquaculture products (EC, 2010). Lack of Codex MRLs for veterinary drugs could be a problem for many developing countries that adopt Codex MRLs as national MRLs. This situation has led FAO/WHO (2004b) to recommend that, for veterinary drugs that have been evaluated by national governments and are legally used in many countries, a comprehensive approach needs to be adopted to expedite harmonization. JECFA evaluation of substances may be constrained by lack of sponsors. FAO/WHO (2004b) recommended that, with the assistance of JECFA and based on national/regional MRLs, an initial list of temporary/operative MRLs could be adopted by the CCRVDF. This list could be made permanent by the CAC, if the national/regional risk assessments are not questioned or if JECFA could establish an ADI using the data used by the country/region to propose a MRL. Substances that do not fulfil these requirements could then be moved to the list of compounds not to be used in food animals.

For veterinary drugs without ADIs/MRLs, regulatory authorities generally adopt a zero tolerance approach. In this situation, as the analytical capability improves, levels that were not detectable by earlier technology become detectable and, hence, reportable. Therefore, independent of any toxicological risk posed by the food product, the residues would attract regulatory action. The countries taking a zero tolerance approach argue that the products are not acceptable because they have evidence of use of a banned drug in animal production and, therefore, represent violation of regulations. For example, in the European Union (Member Organization), the misuse of banned antimicrobials is monitored using an analytical method that has a prescribed minimum required performance limit (MRPL). Liquid chromatography and tandem mass spectrometer (LC-MS/MS) are used to detect residues, and the MRPL for chloramphenicol is 0.3 ppb, and 1.0 ppb for metabolites of nitrofurans (EC, 2003). A national residue control programme needs to be in place as per Council Directive 93/26/EC, and third countries wanting to export to the European Union (Member Organization) need to follow sampling frequencies based on the volume of production.

Table 41 shows the Rapid Alerts due to residues of antibiotics in fish and fishery products that have appeared in the market of the European Union (Member Organization). The major veterinary drugs involved are chloramphenicol, nitrofurans metabolites and malachite green.

TABLE 41

Rapid Alerts due to detection of residues of veterinary drugs in the European Union (Member Organization)

Veterinary drug	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Chloramphenicol	44	102	9	8	1	1	4	2	3	4	178
Nitrofurans (including all metabolites)	0	89	51	27	30	41	31	48	89	11	417
Malachite green	0	2	11	18	50	17	9	2	5	4	118

Following the trade disruptions caused by detection of residues, a Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL was held in 2004. This technical meeting recommended that, for residues of drugs without an ADI/MRL, the CCRVDF should request JECFA to perform and report, if possible, an estimate of the risks associated with the exposure to residues, as such risk estimates would be useful in risk management, and that the CAC should include consideration of cost-benefit and risk comparisons in their risk analysis process (FAO, 2004b). Use of alternate risk management approaches that reflect the toxicological risk of the residue for regulatory analytical methods such as recommended performance level (RPL) or a control strategy chosen by the competent authority were

also recommended (FAO/WHO, 2004b). The meeting further emphasized that the illegal use of veterinary drugs cannot be condoned. A Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance was held in Seoul, the Republic of Korea, in 2006 (FAO/OIE/WHO, 2006). This expert consultation used a risk assessment approach to address the public health impacts of antimicrobial use in aquaculture. The hazards recognized were: (i) development and spread of antimicrobial resistance; and (ii) antimicrobial residues in fish.

3.3.1.1 *Antimicrobials of concern in aquaculture products*

Chloramphenicol: Chloramphenicol was evaluated by JECFA at its twelfth, thirty-second and forty-second meetings and further commented upon at its sixty-second meeting. Dose-related bone marrow depression is the most common outcome in humans, when the daily dose of chloramphenicol is > 4 g (WHO, 2004a). A more serious and unpredictable reaction is aplastic anaemia (with > 50 percent mortality) that can occur at a frequency of 1 in 24 000 to 40 000 courses of treatment with chloramphenicol, but the incidence has been reported to be associated with certain risk factors (WHO, 2004a). Chloramphenicol has ophthalmic use in human medicine, and JECFA evaluation concluded that such use is unlikely to be associated with aplastic anaemia (WHO, 2004b). JECFA also considered the human health risk associated with low levels of chloramphenicol detected in chicken and aquaculture products in the period 2001–03. Based on levels reported by the Food Standards Agency of Ireland, the median concentration in aquaculture products was estimated to be 0.5 ppb. The committee noted that, for preferential eaters of fish and shellfish containing a median of 0.5 ppb chloramphenicol, the exposure would be one order of magnitude lower than exposure from a daily ophthalmic formulation used in human medicine (WHO, 2004b). There are no reported cases of aplastic anaemia associated with ophthalmic use of chloramphenicol. Eckert (2006) carried out a survey of chloramphenicol residues in imported crabmeat in South Australia, Australia, in 2006. Six of 17 samples tested had residues at levels ranging from 0.1 to 0.3 ppb. After reviewing chloramphenicol toxicity data and JECFA review data, the report concluded that the levels found in crab meat were unlikely to cause human health problems. There are no epidemiological records of aplastic anaemia in any country attributable to the residues of chloramphenicol in foods. The levels of chloramphenicol residues found in fish and crustaceans in international trade are generally low (Table 42). The highest number of rapid alerts in the European Union (Member Organization) for chloramphenicol residues was in 2002 (Table 41). In early periods of residue testing, a positive reaction triggered rapid alerts irrespective of the levels detected. To harmonize the reporting by member countries, the European Commission established MRPLs for the assay used for the analytical methods in the detection of residues of banned antimicrobials (EC, 2003). As seen from Table 42, in 2002, about one-third of the alerts were for levels < 0.3 ppb, which was adopted by the European Union (Member Organization) as the MRPL for the assay used for detection of chloramphenicol residues. Rapid alerts in recent years have been triggered by levels exceeding the MRPL.

TABLE 42

Levels of chloramphenicol reported under the Rapid Alert System for Food and Feed for crustaceans in the European Union (Member Organization) in 2002 (n = 92)

Range (ppb)	Number of cases	Comments
< 0.3	32	Lowest level for which alert was issued was 0.07 ppb. In 18 cases, levels were not indicated, but reported as "positive"
0.3–1.0	39	
> 1.0 –5.0	13	
> 5.0	8	Highest level detected was 297 ppb

Nitrofurans: Nitrofurans are synthetic antimicrobials that are rapidly metabolized in animals. The four nitrofurans groups of antimicrobials and their metabolites are shown in Table 43. Furazolidone and nitrofurazone were evaluated by JECFA in 1993 (WHO, 1993). Based on the positive effects of furazolidone in genotoxicity tests *in vitro* and the increased incidence of malignant tumours in rats and mice, JECFA concluded that furazolidone is a genotoxic carcinogen and did not establish an ADI. Nitrofurazone was also evaluated by JECFA in the same meeting, which noted that although this compound is tumourigenic in rats and mice, the tumours produced were benign and restricted to endocrine organs and the mammary gland (WHO, 1993). Mutagenicity studies suggest that nitrofurazone is mutagenic *in vitro* but not *in vivo*. However, JECFA did not establish an ADI, as no-effect levels have not been established for tumourigenic effects. Consequent to JECFA evaluation, use of nitrofurans in animals raised for food was banned in many countries.

TABLE 43
Nitrofurans and their metabolites

Nitrofuran antimicrobials	Metabolites
Furazolidone	3-amino-2-oxazolidinone (AOZ)
Furaltadone	3-amino-5-morpholinomethyl-1,3-oxazolidin (AMTZ)
Nitrofurantoin	1-aminohydantoin (AHD)
Nitrofurazone	Semicarbazide (SEM)

Following detection of residues of nitrofurans in prawns, Food Standards Australia New Zealand (FSANZ) performed a toxicological review and risk assessment (FSANZ, 2005). Data from the Australian Quarantine and Inspection Service (AQIS) and Queensland Health Department showed levels of 3-amino-2-oxazolidinone (AOZ) in the range of 1.1–40 ppb, one sample with 2.2 ppb 3-amino-5-morpholinomethyl-1,3-oxazolidin (AMTZ) and one sample with 8.9 ppb semicarbazide (SEM). FSANZ noted that there are no long-term dietary studies on AOZ that would enable comparison between levels at which AOZ would produce tumours in animals and the level of human dietary exposure to AOZ. Nevertheless, the risk associated with exposure to AOZ was characterized by determining the margin of exposure between the known levels of AOZ residues in prawns for mean and high consumers of prawns and the level of the parent compound furazolidone shown to cause tumours in animal studies. FSANZ noted that there was an approximate 4 millionfold difference between the dietary exposures for high consumers of prawns as compared with the dose shown to cause tumours in animal studies. At mean exposure level, the margin between the dietary exposure and the dose causing tumours in animals was 12 million. FSANZ concluded that, even with a worst-case scenario, the public health and safety risk from nitrofurans residues in prawns was very low.

Data in Table 41 show that rapid alerts for chloramphenicol in the European Union (Member Organization) dropped sharply after 2002. This could be because many fish-exporting countries took measures to control the use of banned antimicrobials in aquaculture and instituted residue control programmes and monitoring of residues in aquaculture products as required by regulations of the European Union (Member Organization). However, the problem with nitrofurans seems to have continued or even to have increased (Table 41). Examination of the data presented in Table 44 suggests that alerts related to the metabolite AOZ, which were highest in 2002, have been declining, while alerts due to SEM have been increasing. Studies conducted in Belgium and the United Kingdom of Great Britain and Northern Ireland revealed that SEM can occur naturally in the shell of crustaceans (Van Poucke *et al.*, 2010) and that the high detection in 2009 could have been due to testing whole animals including

shells. Following these studies, the methodology of testing was changed in the testing laboratories and the SEM alerts came down sharply in 2010 (Table 44).

TABLE 44

Trends in the detection of nitrofuran metabolites in the European Union (Member Organization) in recent years as compared with 2002

Nitrofuran metabolite ¹	Number of cases				
	2002	2007	2008	2009	2010
AOZ	50	21	18	11	2
AMOZ	0	1	0	0	0
AHD	0	0	0	0	0
SEM	0	12	32	76	8
Unspecified	13	0	2	1	1

¹ Metabolites: AZO = 3-amino-oxazolidinone; AMOZ = 3-amino-5-morpholinomethyl-1,3-oxazolidin; AHD = 1-aminohydantoin; SEM = semicarbazide.

Malachite green: Malachite green was evaluated by the Seventeenth Report of JECFA (WHO, 2009). The committee noted that although the available short- and long-term studies point to a NOAEL on the order of 10 mg/kg body weight per day, the study on teratogenicity in rabbits, albeit of low quality, raises concern regarding the potential developmental toxicity of malachite green. It further noted that, as a NOAEL could not be identified, additional studies would be needed to address properly the potential reproductive and developmental hazards of malachite green. Scientific studies indicate that, following ingestion, malachite green is expected to be reduced extensively to leucomalachite green (LMG), primarily by the gastrointestinal microflora, before absorption, and it cannot be ruled out that LMG, the major metabolite of malachite green, induces hepatocellular adenomas and carcinomas in female mice via a mutagenic mode of action. Based on these considerations, the committee considered it inappropriate to establish an ADI for malachite green and did not support the use of malachite green in food-producing animals.

3.3.1.2 Risk management strategy for residues of antimicrobials

The current risk management strategy for antimicrobial residues in aquaculture products is based on the precautionary principle, and there are no epidemiological records of illnesses in fish consumers due to residues. The FAO/OIE/WHO consultation on scientific issues related to non-human usage of antimicrobials held in Geneva, in December 2003, concluded that residues of antimicrobials in foods, under present regulatory regimes, represent a significantly less important human health risk than the risk related to antimicrobial-resistant bacteria in food.

3.3.1.3 Risks associated with selection and spread of antimicrobial resistance

Resistance of bacteria to antimicrobial agents is a complex issue. Some bacteria have intrinsic resistance to certain antibiotics, e.g. most Gram-negative bacteria have intrinsic resistance against penicillin G, all strains of this species being resistant to this antibiotic. This is because of the double membrane structure of the cell wall in these bacteria. There are also situations where bacteria that are normally susceptible to an antimicrobial agent are not adversely affected. For example, microbial cells in biofilms show resistance compared with planktonic cells owing to protection provided by the extracellular matrix composed of polysaccharides or proteins.

Initially susceptible populations of bacteria may become resistant by mutation or by acquiring from other bacteria genetic elements that encode resistance, and the latter might occur through one of the modes of gene transfer, viz. transformation, conjugation or transduction. There are a number of mechanisms by which bacteria may resist an antimicrobial agent including enzymatic degradation (β -lactamases, chloramphenicol

acetyl transferase), alteration of specific drug receptors (e.g. ribosomal proteins, gyrase A or gyrase B proteins), change in membrane permeability (e.g. alterations in porins), increased pumping out of drugs (e.g. efflux pumps), or changes in metabolic pathway (e.g. bypassing folic acid synthesis).

Recent molecular biological studies provide insights into the evolution and ecology of antibiotic resistance genes. Tetracycline resistance is mediated by ribosomal protection protein (RPP) in Gram-positive and Gram-negative bacteria. Two recent publications (Kobayashi *et al.*, 2007; Aminov and Machie, 2007) provide evidence to show that at least some antibiotic resistance genes have a long evolutionary history of diversification that began well before the antibiotic era. Kobayashi *et al.* (2007) note that RPPs were derived through duplication and divergence of GTPase, before the divergence of the three superkingdoms: Bacteria, Archaea and Eukarya. This suggests that the extant function of RPPs occurred even before evolution of *Streptomyces* that produce tetracyclines. They suggest that RPPs evolved independently of tetracyclines and that they possibly serve a function other than antibiotic resistance. β -lactamases are enzymes involved in resistance to the penicillin group of antibiotics. Fevre *et al.* (2005) provided evidence to show that β -lactamase genes in *Klebsiella oxytoca* had been evolving for more than 100 million years in this host, without concomitant evolution of an antimicrobial resistance phenotype. In addition to being involved in hydrolysis of the β -lactam ring, metallo- β -lactamases are involved in various basic cellular processes such as hydrolysis, DNA repair, RNA processing, and these enzymes can be found in all three domains of life, i.e. Bacteria, Archaea and Eukarya (Garau, Di Guilmi and Hall, 2005). The ancient evolution of antibiotic resistance genes is further supported by observation of antibiotic resistance in bacteria trapped in deep Greenland glacier ice cores at least 120 000 years ago (Miteva, Sheridan and Brenchley, 2004).

Although antibiotic resistance genes may emerge as a process of natural genetic changes occurring in bacteria, the presence of antibiotics would exert selective pressure favouring resistant bacteria and their spread. Multiple antibiotic resistance in bacteria causing human infections is a great public health concern. The widespread use of antibiotics in different sectors such as animal husbandry, agriculture and human medicine has contributed to the selection and spread of antibiotic-resistant bacteria in the environment. Antibiotic resistance genes can spread among unrelated bacteria without any phylogenetic, ecological or geographical barriers. The Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance held in 2006 identified two types of hazard in respect of antimicrobial resistance:

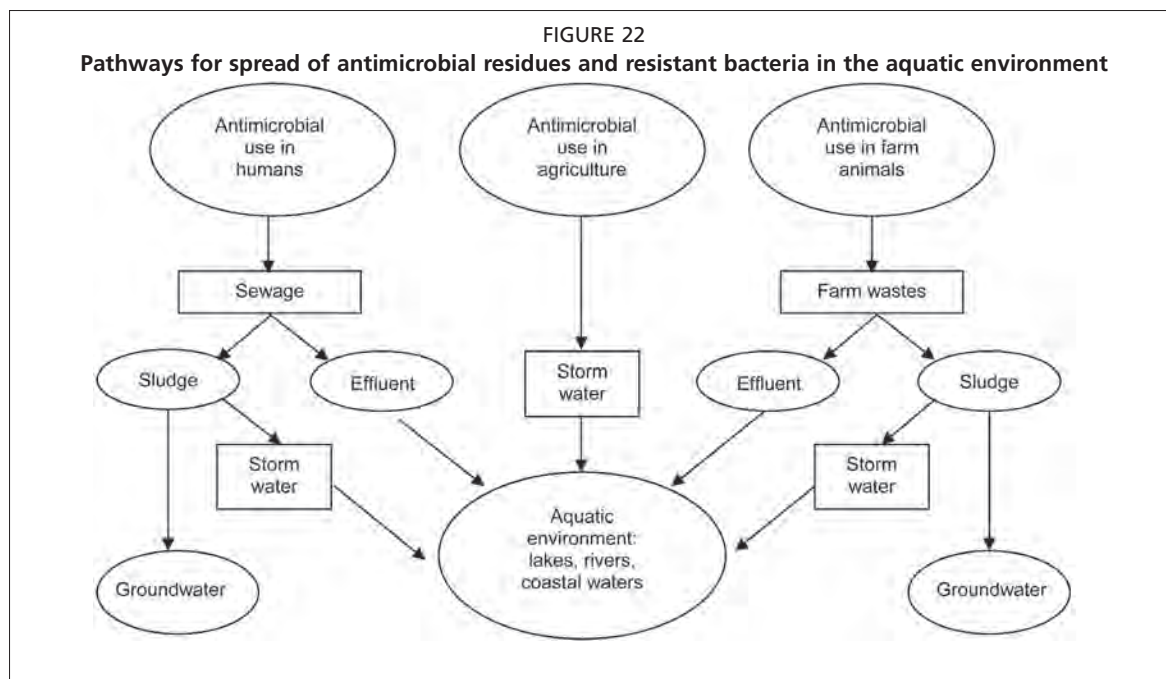
- Development of acquired resistance in bacteria in aquatic environments that can infect humans. This can be regarded as a direct spread of resistance from aquatic environments to humans.
- Development of acquired resistance in bacteria in aquatic environments whereby such resistant bacteria can act as a reservoir of resistance genes from which the genes can be further disseminated and ultimately end up in human pathogens. This can be viewed as an indirect spread of resistance from aquatic environments to humans caused by horizontal gene transfer.

The consequences of antimicrobial resistance in bacteria causing human infections could include increased severity of infection and increased frequency of treatment failures (FAO/OIE/WHO, 2006). However, there are no recorded cases of human infections caused by antibiotic-resistant bacteria from aquaculture products.

There are few human pathogenic bacteria that are commonly found in the aquatic environment (e.g. *Vibrio parahaemolyticus*, *V. vulnificus*, *V. cholerae*, motile *Aeromonas* spp., and *Edwardsiella tarda*). Antibiotic resistance that cannot be linked to the use of antimicrobials in aquaculture may be found in these aquatic bacteria. Baker-Austin *et al.* (2008) found antibiotic resistance in *V. parahaemolyticus* isolated

from water and sediment along the coasts of Georgia and South Carolina, the United States of America, and resistance frequency was slightly reduced among virulent strains compared with non-virulent strains. Baker-Austin *et al.* (2009) examined antibiotic resistance in *V. vulnificus* from different sites and found no difference in antibiotic resistance frequency in isolates from pristine and anthropologically impacted areas. They suggested that the resistance traits were naturally derived rather than from human-derived sources. A recent FAO/WHO risk assessment has shown that the risk of transmission of cholera through warmwater shrimp in international trade is very low (FAO/WHO, 2005b). Motile *Aeromonas* spp. and non-O1 *V. cholerae* are rarely involved in gastrointestinal infections that are mostly self-limiting, and such infections do not require antibiotic therapy.

The indirect spread of antibiotic resistance from aquatic bacteria and human pathogens has been considered a possible hazard. A number of investigators have reported increased prevalence of bacteria carrying antibiotic resistance genes in fish/shrimp ponds and in water and sediments surrounding aquaculture sites in Japan (Kim, Nonaka and Suzuki, 2004), Europe (Kerry *et al.*, 1996; Schmidt *et al.*, 2000), the United States of America (Herwig, Gray and Weston, 1997), South America (Miranda and Zemelman, 2002), China (Dang *et al.*, 2009) and Southeast Asia (Karunasagar, Venugopal and Karunasagar, 1984; Le, Munekage and Kato, 2005). Although experimental transfer of antibiotic resistance from bacteria from fish-pathogenic bacteria to human-gut-associated *E. coli* has been demonstrated (Kruse and Sorum, 1994), a link between antibiotic resistance in aquatic bacteria and human pathogens in nature is yet to be clearly established. Often, similarity in genetic elements is taken as evidence of transfer, but one cannot be sure in which direction the gene flow has occurred, considering that hospital effluents also discharge antibiotic-resistant bacteria to the aquatic environment. Some authors (e.g. Cabello, 2006) have tried to link the antibiotic resistance seen in *V. cholerae* involved in the cholera outbreak in Latin America in 1991 with bacteria present in shrimp farms in Ecuador. However, Smith (2007) presented evidence that resistance plasmids found in these bacteria were earlier reported from pandemic *V. cholerae* strains in other countries and concluded that no link to the pool of resistance genes in the aquaculture environment could be established. Conclusions based on similarity of genetic determinants found in aquatic bacteria and human pathogens need to be evaluated carefully owing to the fact that the aquatic environment receives effluents from various sectors of antimicrobial use, e.g. human medicine (hospital effluents), agricultural use, animal husbandry and aquaculture (fish-farm effluents). Thus, the water source used in aquaculture may be contaminated with antibiotic residues or antibiotic-resistant bacteria derived from different sectors (Figure 22). FAO (2008a) noted that a risk analysis of the release of human and animal effluents into aquatic environments serving as water sources for aquaculture needs to be performed, particularly with respect to the antimicrobials identified as critically important by WHO and OIE. Such a risk analysis would determine the appropriate management options through which improved effluent management measures should be implemented (e.g. measures dealing with hospital effluents). Thus, the issue of antimicrobial resistance cannot be addressed for one sector (e.g. aquaculture) alone, but requires a comprehensive approach involving all sectors of antimicrobial usage.



3.3.2 Industrial (organic) contaminants (Horst Karl and Jörg Oehlenschläger)

Industrial contaminants in the aquatic environment and biota include a wide range of compounds that have entered the sea mainly by anthropogenic activities.

Most of these compounds are organic chemicals produced for a variety of different applications. The majority of these substances were considered useful products before their negative impact on the environment and biota was noticed.

The range of products that later turned out to be hazardous to humans and to the biota includes herbicides and pesticides for agriculture, such as toxaphene, chlordane or 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT). Between 1950 and 1963, DDT was the most important pesticide for malaria vector control caused by *Anopheles* mosquitoes, and it is still applied effectively in some areas of the world for this purpose. In 2001, DDT was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants with the aim that the production and use of DDT should be eliminated worldwide, except for restricted and controlled use as disease control vector where alternatives were not available.

Compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have been used in large quantities as additives and fire retardants in a range of consumer and commercial products, including plastics, electronics, textiles, car seats, polyurethane foams, and fire extinguishers.

Furthermore, breakdown products such as dichlorodiphenyldichloroethane and dichlorodiphenyldichloroethylene from DDT or by-products generated during production, such as hexachlorocyclohexane from lindane production, have found their way into the aquatic system.

Some other compounds such as dioxins that have never been produced for any industrial purpose are formed as unwanted by-products from certain industrial processes (e.g. metallurgical industry) and combustion processes such as waste incineration. Dioxins can also be formed during natural processes such as forest fires or volcanic eruptions.

The list of industrial contaminants has to be extended by industrially produced household chemicals (e.g. musk fragrances, and nonylphenol) or, most recently, by

the class of perfluorinated alkylated substances, which have found a widespread use as protective coatings for carpets, papers and fabrics.

Organic chemicals can have a direct toxic effect on fish or negatively influence their reproduction ability. Other compounds, especially the group of persistent organic pollutants (POPs), accumulate in fish tissue and result in human exposure to these compounds when fish is used as a foodstuff.

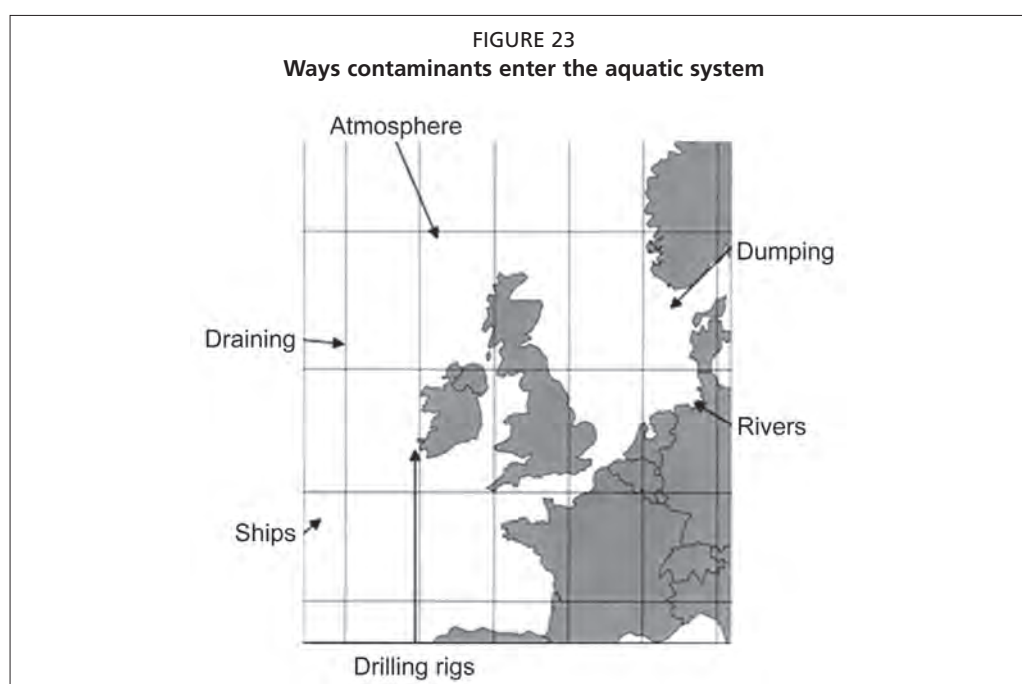
3.3.2.1 Entry of industrial contaminants into the aquatic system

The input of industrial contaminants into the aquatic system occurs via the atmosphere, rivers, by direct dumping, from leakages of drilling rigs, from ships and from draining of contaminated areas (Figure 23).

Organic contaminants enter the marine environment via direct input from ships and drilling rigs, by dumping of sludge from sewage plants, by draining into the sea of chemical used in agriculture, via rivers from large urban populations and via atmospheric input from emissions through thermal processes.

Long-range transport via the atmosphere results in a global distribution of organic contaminants. The global distribution of chemicals depends on various factors such as mobility and persistence of the substance, area of release and environmental conditions including temperature, light and precipitation.

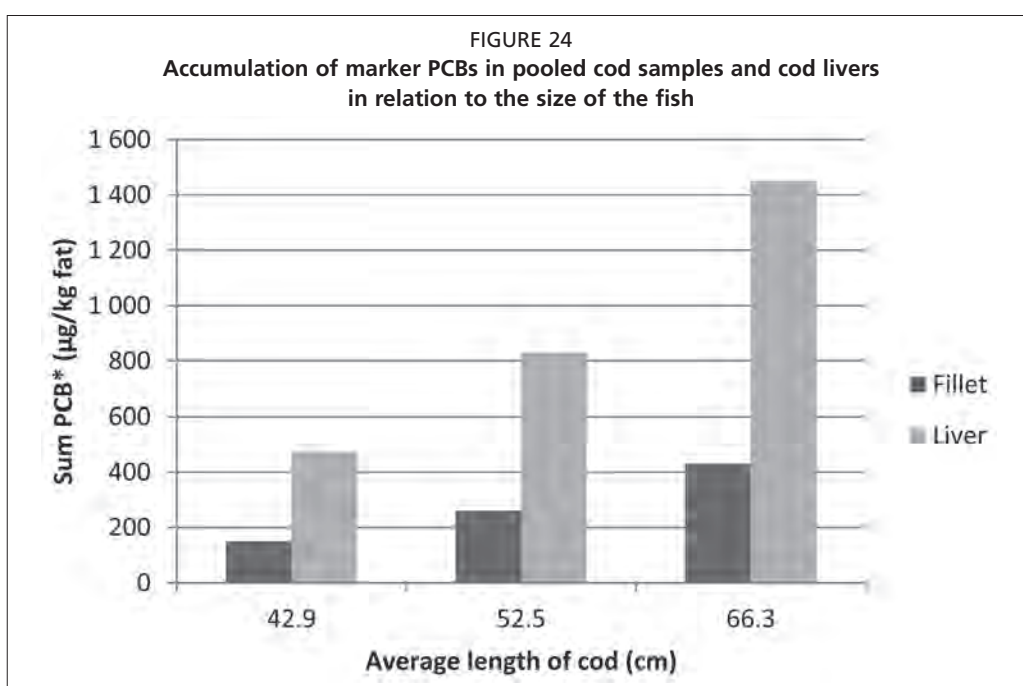
Generally, most of the worldwide industrial production facilities are established in the Northern Hemisphere, resulting in a higher emission of industrial contaminants in this part of the world compared with the Southern Hemisphere. Several studies have shown that the higher releasing rate of organic contaminants leads to higher concentrations in fishes of the Northern Hemisphere. Within the Northern Hemisphere, emitted chemicals are often transported via the atmosphere from the industrial belt to the polar region, where they condense in the cold climate and finally reach the fish, birds and mammals of remote and clean areas such as the Barents Sea or waters around Greenland. The same situation also occurs in the Southern Hemisphere and explains the regular detection of organic contaminants in Antarctic fishes and mammals. Long-range atmospheric transport together with their persistence is responsible for the ubiquitous occurrence of organic contaminants such as PCBs, dioxins and PBDEs in waters, sediments and biota.



3.3.2.2 Uptake by fish

The uptake of contaminants by fish occurs via diet and from the water via gills and skin. In farmed fish, whose lifespan is short compared with wild-living fish, the uptake occurs mainly via feed.

Most of the organic contaminants detected in fish are lipophilic and stored in the fat tissue of muscle (in fatty-fish species) and liver (in lean-fish species). Inorganic contaminants are mainly stored in the intestines, liver and kidneys but are also found in the muscle often bound to proteins. Bottom-dwelling fish species and bottom feeders are more exposed to contaminated sediments than are pelagic fish species. However, levels of contaminants in bottom-dwelling fish are not always higher than those in pelagic fish. The concentrations depend on the size or age, on the fishing ground and on physiological characteristics (biological cycle) of the fish. The deposition within the fish is of vital interest concerning the possible exposure of the edible part.



Note: * Sum PCB = PCB 52 + 101 + 138 + 153 + 180.

Figure 24 demonstrates the age/size-dependent increase in PCBs in the fat tissue of cod muscle and cod liver. As a result of bioaccumulation, the concentrations in the liver are 3–4 times higher than in the fat phase of the fillets. Generally, older fish are larger and will eat larger prey species, which results in an accumulation of higher amounts of contaminants over a longer period of their life span. The liver acts as a detoxification organ and can be considered as an organ accumulating lipophilic contaminants.

The presence of chemical contaminants in seafood is also highly influenced by geographic locations (fishing grounds). Fish from rivers, lakes or coastal ocean areas with a high input of wastewater and/or effluents from industrial processes are often more contaminated than fish from the open oceans and may exceed legal maximum limits set for certain pollutants.

For seafood from aquaculture, a correlation exists between contaminant level of the feed and concentrations found in the edible part. While it is not possible to control the diet of wild fish, contaminant concentrations in farmed fish can be influenced by the composition of the feed.

An important part of feed for carnivorous species such as trout and salmon is fishmeal and fish oil. Fishmeal and fish oil are known to be the major source of dioxin-like compounds in fish feed. By modifying the composition of the feed, a significant reduction in the uptake of dioxin-like compounds via feed is possible.

The fat tissue is the main deposit for lipophilic contaminants in fish. The fat content varies widely with the biological cycle and the species. All species increase their lipid content during the feeding seasons prior to maturation as an energy reservoir for the development of gonads.

Fatty species such as salmon, herring and mackerel store the lipids in the edible muscle tissue, whereas lean species such as Atlantic cod, Alaska pollock and saithe store lipids in the liver. Consequently, the edible part of fatty-fish species contains more lipophilic contaminants compared with lean species. Moreover, the contaminant level can depend on the time of the year a fish is caught (as a function of the state of maturity).

3.3.2.3 *Current situation*

A modest concentration of industrial contaminants is ubiquitous in the environment and the aquatic system but risks from chemical contaminants in commercially harvested fish and shellfish are low and not a principal problem, with a few exceptions. Large specimens of predator fish such as shark or tuna and swordfish can reach elevated levels of some heavy metals (mercury) owing to life-long bioaccumulation via the food chain from geogenic sources. This problem is not related to industrial and human activities.

The levels of organochlorines in most fish intended for human consumption are low and probably below levels likely to affect human health adversely. However, some coastal areas, lakes and rivers can be polluted, and consumption of substantial quantities of oily fish from these areas can be a cause of health concern, especially for infants, young children and pregnant women.

At the request of the European Commission, the Scientific Panel on Contaminants in the Food Chain⁴ evaluated the risks and benefits of human consumption of fish and concluded that fish consumption, especially of fatty fishes as a source of LCn3PUFAs, benefits the cardiovascular system, foetal development and is suitable for secondary prevention in coronary heart disease. It also concluded that fish contributes to the dietary exposure to contaminants such as methylmercury, persistent organochlorine compounds, brominated flame retardants and organotin compounds, but only methylmercury and the dioxin-like compounds are of health significance because high-level consumers of certain fish species may exceed the provisional tolerable weekly intake (PTWI) established by Scientific Committee of the European Community. In its opinion, intakes of the other contaminants are not a health concern as they do not contribute significantly to total dietary exposure.

When discussing human dietary exposure to contaminants via fish, it has to be taken into account that considerable differences can exist between PTWI values derived by the European Food Safety Authority (EFSA) and the United States Environmental Protection Agency (EPA) reference doses.

3.3.2.4 *Dioxins and dioxin-like polychlorinated biphenyls*

A recent FAO Fact Sheet⁵ provides an overview about dioxins in the food chain. Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), collectively referred as to dioxins, are unwanted and often unavoidable by-products from a number of industrial and thermal processes. They are very persistent chemicals that are ubiquitous in the environment, but they are also present

⁴ www.efsa.europa.eu/en/efsajournal/pub/2985.htm

⁵ Available at: www.fao.org/ag/againfo/home/en/news_archive/2009_IN_dioxin.html

in low concentrations in food. They are lipophilic compounds and accumulate in the food chain.

Dioxin-like polychlorinated biphenyls belong to the group of PCBs that were manufactured between the 1930s and the late 1970s for use in electrical equipment and other purposes. There are 209 PCB congeners, 12 of which exhibit similar toxic properties to those of the toxic dioxins and are, therefore, called “dioxin-like PCBs” (dl-PCBs). Of the identified 419 dioxins and dl-PCB compounds, which have a dioxin-like chemical structure, only about 29 are considered to have significant toxicity, with 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) being the most toxic. The highest levels of these compounds are found in soils, sediments and food, especially in dairy products, meat, fish and shellfish. Very low levels are found in plants, water and air.

Dietary intake is the major route of dioxin exposure of humans, contributing more than 90 percent of the daily intake of these compounds. The toxicity of PCDDs, PCDFs and dl-PCBs is expressed using toxic equivalence factors (TEFs), representing the relative toxicity of the congener in relation to the most toxic dioxin congener, TCDD with a TEF of 1.0. Today, the most common toxicity factors applied are the WHO-TEFs, proposed in 1998. Recently, WHO experts have re-evaluated the toxicity of the single dioxin and dl-PCB congeners and slightly modified the TEFs for some congeners.

Multiplying the measured concentration of each congener in a sample by its corresponding WHO-TEF, the individual toxicity equivalents (WHO-TEQ) are obtained. The sum of all WHO-TEQs gives the total WHO-TEQ value of a sample.

The available toxicity data allowed WHO to establish a toxicity equivalents value of 1–4 pg WHO-TEQ per kilogram of body weight. The European Scientific Committee for Food fixed a tolerable weekly intake (TWI) of 14 pg WHO-TEQ per kilogram of body weight for dioxins and dl-PCBs.

To reduce human exposure to dioxins and dl-PCBs, maximum levels have been set for various foodstuffs and animal feed. The maximum levels are set to 4 pg WHO-PCDD/F-TEQ/g wet weight (w.w.) for fish (including eel) and 8 pg/g w.w. for the sum of WHO-PCDD/F-TEQ and WHO-PCB-TEQ (WHO-TEQ) for fish and 12 pg WHO-TEQ/g w.w. for eel, respectively.

Results from a large number of surveys have shown that the actual contaminant levels of most fish and fishery products are below these limits.

Total WHO-TEQ concentrations (PCDD/F+dl-PCB) of lean-fish species such as Atlantic cod, Alaska pollock or haddock are typically below 0.5 pg/g w.w. Fish with a moderate fat content (< 5 percent) such as hake, plaice or sea bream range between 0.5 and 2 pg/g w.w., and levels in fish with a higher fat content such as mackerel, herring or farmed salmon vary between 1 and 3 pg/g w.w. High concentrations have been reported in eels from rivers with industrial activities and in old fatty-fish from the eastern Baltic Sea. On the other hand, very low concentrations have been found in farmed shrimps from Asia and wild Pacific salmon species from Northern America.

3.3.2.5 “New” contaminants

Brominated flame retardants: Brominated flame retardants (BFRs) have been widely added to a variety of commercial and household products (plastics, polyurethane foam, textiles and electronic items) in order to improve their fire resistance and they are now appearing as contaminants in food (De Boer, 2008). The BFRs can be divided into three major types depending on their use: tetrabromobisphenol A (TBBP-A), hexabromocyclododecane (HBCD) and polybrominated diphenyl ethers (PBDEs). The total world market demand for BFRs was more than 200 000 tonnes in 2001. Both HBCD and PBDEs have shown potential for biomagnification in fish via the food chain owing to their lipophilicity and persistence.

An increasing trend in PBDEs has been detected in human breast milk, human adipose tissue and in fish over the last 20 years, which is in contrast to the observed decline of other chlorinated pesticides.

Dominating congeners are the lower brominated tetra-, penta- and hexa-BDEs. Typical concentrations in wild marine fish species range between 10 and 300 ng Σ PBDEs per gram of fat, but results are difficult to compare, often because different single congeners have been measured.

Farmed salmon contains higher levels of PBDEs compared with wild salmon.

First studies indicate that the HBCD content in fish ranges between < 1 and 200 ng/g fat and that the concentrations depend on the fishing ground and on the species. However, data on HBCD are still too limited to give an overview on contaminant levels in fish. To reduce the impact of PBDEs to the environment, the use of penta- and octa-mix PBDE formulations has been recently prohibited in the European Union (Member Organization).

Perfluorinated alkylated substances: Perfluorinated alkylated substances (PFASs) have recently been found to be distributed in the environment all over the world. Major applications of PFASs include coating of paper and food packaging, and impregnation of textiles and carpets and as surfactants in the paint and spray industry. They are persistent, have a tendency to bioaccumulate and are of health concern. They are heat resistant and water and oil repellent. In the marine biota, primarily perfluorooctane sulphonate, long-chain perfluorocarboxylic acids (PFCAs) with carbon atoms between 7 and 14, but also other fluorinated compounds have been detected. The behaviour of this class of chemicals is different to the lipophilic halogenated organic pollutants and, therefore, exposure routes are difficult to assess.

Data on concentrations in fish vary considerably owing to analytical uncertainties, and additional work is needed to improve the reliability of analytical techniques.

Several time-trend studies have shown that contaminant levels of most of the organic contaminants in fish and fishery products have declined in the last 20 years. However, more time is necessary to further reduce the contamination of seafood. Measures should be directed to eliminating existing sources, to minimizing releases, and to ensuring improved control disposals and prevention of the release of new contaminants into the environment.

3.3.3 Environmental inorganic contaminants (Jörg Oehlenschläger and Horst Karl)

3.3.3.1 Introduction

Many elements, such as selenium, iodine, fluoride, iron and phosphorous, which are present in fish, crustaceans and molluscan shellfish, are essential for humans at low concentrations (Reilly, 2004; Oehlenschläger, 2010). However, some of them can be toxic at elevated levels. Other elements, such as mercury, cadmium and lead, have no known essential biological function and are toxic even at low concentrations when ingested over a long period. As a result, many consumers regard any presence of inorganic elements in fish as a hazard to health. However, the presence of these elements in the aquatic environment predates human evolution. In contrast to the inorganic elements, organic contaminants, which are industrially produced anthropogenic xenobiotics, have only recently been introduced into the environment by planned human activity. The only exception is the dioxins, which are produced as a result of combustion, industrial accidents and natural processes.

The presence and concentration of heavy metals in the environment, more specifically in the aquatic environment and in its biota, namely in animals and plants that are used as human food, are based on both natural and anthropogenic sources. Natural (background) concentrations of these elements are present in the world's oceans and freshwater reservoirs owing to volcanoes, geological anomalies and

geothermal events, but considerable anthropogenic pollution started with the period of the industrial revolution. Later, acidic rain, as a result of industrial pollution, mobilized heavy metals from minerals and contributed more to the overall concentration.

As mentioned above, fish and other seafood have always contained certain amounts of heavy metals as a consequence of living in an aquatic environment. The distribution between the natural background concentration of heavy metals and anthropogenic heavy metals in fish varies depending on the element, the species and the area of capture. In the open seas, which are still almost unaffected by pollution, fish mostly carry just the natural burden of heavy metals. In moderate or heavily polluted areas, such as those seas that do not have sufficient exchange with the world oceans (e.g. the Baltic Sea and the Mediterranean Sea), in estuaries, in rivers, in lakes and especially in places with close vicinity to industrial activities, the heavy metal concentrations found in seafood exceed the natural concentrations (Celik and Oehlenschläger, 2007).

There is a vast literature on the content of toxic heavy metals in fish, crustaceans, molluscs and seaweed (e.g. Alfonso *et al.*, 2007; Besada *et al.*, 2009; Burger *et al.*, 2007; Fabris, Turoczy and Stagnitti, 2006; Julshamn *et al.*, 2004; Kaneko and Ralson, 2007; Kikuchi *et al.*, 2002; Knowles, Farrington and Kestin, 2003; Llobet *et al.*, 2003; Plessi, Bertelli and Monzani, 2001; Rasmussen, Nettleton and Morrissey, 2005; Sidoumou *et al.*, 2005; Storelli, Storelli and Marcotrigiano, 2001; Ysart *et al.*, 2000). Some of these papers deal with concentrations of heavy metals that are unusually high owing to anthropogenic activities and are found in areas where an accumulation is favoured by the natural conditions (insufficient water exchange, shallow waters, estuaries, rivers, inshore waters, etc).

Other publications investigate organs and components of the body that accumulate and store heavy metals, while the muscle tissue (the fillet), which is generally the only part of the fish that is actually eaten by humans, has been of lesser interest because of its generally low burden. Only a small amount of information is available about the heavy metal content in the edible part of the food fish that are caught in the open ocean and commonly consumed by humans but which contain only natural background concentrations in their muscles (Oehlenschläger, 2002). A short review of the analytical methods in use for toxic elements has recently been published (Capar, Mindak and Cheng, 2007). Useful information about the analysis of contaminants in edible aquatic resources can also be found in Kiceniuk and Ray (1994), and information about the speciation of element traces is provided in Ebdon *et al.* (2001).

Aluminium, arsenic, tin, cadmium, lead and mercury are addressed in this chapter, which also gives information on the current situation. Some recent papers about risk assessment and seafood consumption are also reviewed.

The FDA (2009) has distributed information about a quantitative risk and benefit assessment of consumption of commercial fish.

3.3.3.2 *Aluminium*

Aluminium is present in seafood as a result of its high concentration in nature. Acid rain decreases the pH of the soil, increasing the transportation of aluminium in subterranean water. Information about the possible toxic effects of aluminium has increased in recent years. There is a possible correlation between high aluminium concentrations in human tissues and the appearance of certain neurodegenerative disorders, such as Alzheimer's disease. The aluminium content in food, including seafood, consumed in Spain (Lopez *et al.*, 2000) and the aluminium content in seafood from the North East Atlantic (Ranau, Oehlenschläger and Steinhart, 2001) has been investigated. In the Spanish study on aluminium in fish from coastal Mediterranean waters, the aluminium concentrations ranged from 1.36 mg/kg to 6.6 mg/kg w.w.. The mean concentration in fish amounted to 3.3 mg/kg, in crustaceans to 4.3 mg/kg and in molluscs to 2.8 mg/kg. The study on North Atlantic fish species

showed much lower aluminium concentrations. In species caught in the North Sea, the range was from 0.05 mg/kg to 0.27 mg/kg; while in species from the North East Atlantic Ocean; the range was from 0.03 mg/kg to 0.14 mg/kg. The aluminium concentrations in fish from the Barents Sea, Greenland waters and Baltic Sea were in the same range. Elevated aluminium concentrations were only detected in cod (0.29 mg/kg), pollock (0.28 mg/kg), haddock (0.94 mg/kg) and ling (0.29 mg/kg) caught in coastal Norwegian waters in the vicinity of an aluminium smelter. It seems that the aluminium content in fish from coastal Mediterranean waters is about 10 times higher than that in fish from the North Atlantic.

3.3.3.3 Arsenic

The environmental origin, occurrence and impact of arsenic on human health have been reviewed by Mandal and Suzuki (2002). A critical review of the methods and applications for the determination of arsenic species has been presented by Francesconi and Kuehnelt (2004). The risk assessment of arsenic in seafood for human beings and/or the toxicity of arsenic for humans have been described in review papers (De Gieter and Baeyens, 2005; Borak and Hosgood, 2007; Lorenzana *et al.*, 2009). The fact that high concentrations of inorganic arsenic can lead to intoxication was described by Amster, Tiwary and Schenker (2007), who reported a case of potential arsenic toxicity resulting from a herbal kelp supplement. The major proportion of arsenic in seafood is in the organic form, e.g. as arsenobetain, arsenocholine or arsenosugars. A minor fraction is inorganic arsenic, which is the toxic form of this element. Table 45 shows total arsenic concentrations and concentrations of inorganic arsenic as well as the proportion of inorganic arsenic based on some recently published papers (Baeyens *et al.*, 2009; De Gieter *et al.*, 2002; Sloth, Julshamn and Lundbye, 2005; Schoof and Yager, 2007; Schoof *et al.*, 1999; Greene and Crecelius, 2006; Peshut, Morrison and Brooks, 2008; Fabris, Turoczy and Stagnitti, 2006).

Table 45 shows that the proportion of inorganic arsenic rarely exceeds 1 percent of total arsenic concentration, and that the total arsenic concentration in marine animals varies considerably among species. Flatfish species have a higher concentration of arsenic compared with other finfish species. De Gieter *et al.* (2002) found that the highest total arsenic concentrations in North Sea fish were found in lemon sole, dogfish, ray and witch, with average total arsenic concentrations exceeding 20 mg/kg w.w. These species also contained the highest amount of toxic inorganic arsenic (> 0.1 mg/kg w.w.). More than 2 percent of inorganic arsenic has been found in sea bass, ling, John Dory, pouting, dab and brill.

Sloth, Julshamn and Lundbye *et al.* (2005) demonstrated that, in feed used for aquaculture total, an average arsenic concentration of 6 mg/kg on a product weight basis was found. The feeds contained an average of 40 µg/kg inorganic arsenic, which amounted to 0.5 percent of total arsenic. The authors recommend that further legislation should be based on the toxic inorganic arsenic content rather than on total arsenic concentration.

Inorganic arsenic in concentrations of up to 69.5 mg/kg dry weight (Besada *et al.*, 2009) have been found in the edible seaweed *Hizikia fusiforme* (commercial name Hiziki), making it the marine food item with the highest concentration of inorganic arsenic. In 2004, the United Kingdom Food Standards Agency advised consumers not to eat *Hizikia fusiforme* because of its high level of inorganic arsenic (Anon, 2004).

TABLE 45

Total arsenic, inorganic arsenic and proportion of inorganic arsenic in marine species

Location	Species	Total As range or mean (mg/kg wet weight)	Inorganic As range or mean (mg/kg wet weight)	Inorganic As (%), range or mean
American Samoa	<i>Panulirus</i> sp.	19.8–97.4	0.009–0.03	0.02–0.2
American Samoa	<i>Mugilidae</i> sp.	0.32–0.94	0.09–0.18	10–37
Mid-Atlantic region, United States of America	<i>Paralichthys dentatus</i>	0.95–3.33	0.0005–0.03	
Mid-Atlantic region, United States of America	<i>Micropogonias undulates</i>	0.48–0.80	0.0006–0.03	
Mid-Atlantic region, United States of America	<i>Morone saxatilis</i>	0.36–2.19	0.002–0.03	
North Sea	<i>Squalus acanthias</i>	40.7	0.24	0.59
North Sea	<i>Conger conger</i>	2.37	0.028	1.18
North Sea	<i>Gadus morhua</i>	5.1	0.068	1.33
North Sea	<i>Lophius piscatorius</i>	8.5	0.079	0.93
North Sea	<i>Limanda limanda</i>	10.2	0.19	1.86
North Sea	<i>Pleuronectes platessa</i>	13.8	0.18	1.3
North Sea	<i>Microstomus kitt</i>	39.7	0.23	0.58
North Sea	<i>Solea solea</i>	14.5	0.23	1.45
North Sea	<i>Cancer pagurus</i>	38.7	0.29	0.75
Australia	Abalone	7.7	0.01–0.06	0.3–0.9
Australia	Snapper	4.7	0.01–0.03	0.2–0.3
Australia	Lobster	50.7	0.01–0.1	< 0.1–0.2

3.3.3.4 Tin

In the last four decades, the extensive use of organometallic tributyltin compounds in antifouling paints for ships, slime control in paper mills, disinfection of circulating industrial cooling water and the preservation of wood has created a global pollution problem. Organotins for agricultural, industrial and biomedical applications are produced at an estimated rate of approximately 60 000 tonnes per year. It is now well established that at very low concentrations tributyltin causes reproductive and developmental effects on a wide diversity of aquatic organisms, especially on molluscs. The United States EPA has set the saltwater chronic criterion for tributyltin at a value as low as 1 ng/litre (US EPA, 2002). The widespread application of organotin compounds has increased the possibility of their intake by human beings even in regions where this was not expected (Sheikh *et al.*, 2007). Triorganotin compounds are the most toxic and affect a variety of biochemical and physiological systems. Trialkyltin and triphenyltin compounds interfere with haem metabolism as well as the cardiovascular system, cause a fall in blood pressure, alter blood composition and result in a decrease in organ–heart ratios in rats and mice (Guerin *et al.*, 2007; Nath, 2008; Antizar-Ladislao, 2008). Table 46 gives a short overview of organotin concentrations in aquatic animals in different parts of the world (Guerin *et al.*, 2007; Barroso, Mendo and Moreira, 2004; Keithly, Cardwell and Henderson, 1999).

Guerin *et al.* (2007) found that marine fish contained an average of 1.6 µg of tributyltin per kilogram (range: 0–11 µg), bivalves and molluscs 3.1 µg (0.6–6.7 µg), cephalopods 4.5 µg (0.7–10 µg) and crustaceans 3.0 µg (0–10 µg), respectively.

TABLE 46

Organotin and tributyltin concentrations in marine species from different parts of the world

Species or group	Region	Σ organotin compounds ($\mu\text{g/kg}$ wet weight)	TBT ($\mu\text{g/kg}$ wet weight)
Cod	France	3.4	0.7
Hake	France	4.5	1.2
Mackerel	France	8.8	2.4
Sardine	France	6.4	1.5
Halibut	France	23.2	10.5
Sea bass	France	11.0	4.3
Swordfish	France	19.2	7.7
Tuna	France	7.3	1.9
<i>Mytilus galloprovincialis</i>	Portugal		11–789 (dry weight)
<i>Mytilus galloprovincialis</i>	Republic of Korea		115
<i>Scomber japonicus</i>	Republic of Korea		12
<i>Loligo vulgaris</i>	France		655
<i>Thunnus thynnus</i>	France		56
<i>Crassostrea gigas</i>	United States of America		72
<i>Loligo chinensis</i>	Singapore		12
<i>Clupea harengus</i>	Sweden		36
<i>Scomber australasicus</i>	Australia		13
<i>Mytilus edulis</i>	Canada		5.6
<i>Clupea harengus</i>	United Kingdom		11
<i>Pleuronectes platessa</i>	United Kingdom		1.9

3.3.3.5 Cadmium

Cadmium is one of the most toxic heavy metals for human beings. It is widely distributed in the aquatic environment, and bioaccumulation of cadmium up the food chain by some aquatic organisms is widely recognized. Cadmium content in the edible part of fish is generally very low, while fish deposit cadmium in organs such as the kidney and liver. These organs can be heavily contaminated and should not be consumed.

The situation in invertebrates, such as molluscs and crustaceans, is different. Molluscs, especially cephalopods, are active cadmium accumulators. Cephalopods can store huge amounts of cadmium in their intestines while the muscle is still low in cadmium (Storelli, Barone and Marcotrigiano, 2005; and Table 47). To prevent ingestion of cadmium-contaminated seafood, cephalopods have to be gutted immediately after catch or harvest. Mussels show a similar affect, however, on a low level. For this reason, they also have to be checked regularly for their cadmium content. Cadmium concentrations for different marine species are given in Table 47. Table 47 shows that the cadmium concentration in the edible part of fish species (fillet) is low, but molluscs have a high potential to accumulate cadmium, especially in their digestive glands.

Llobet *et al.* (2003) reported an average cadmium concentration in fish and shellfish from Catalonia, Spain, of 0.037 mg/kg w.w. in a study about heavy metal intake by children, adolescents, adults and seniors. Storelli, Storelli and Marcotrigiano (2001) analysed cadmium in three species of algae from the Apulian coast (Italy) and found concentrations ranging between 0.20 and 0.72 mg/kg on a dry weight basis. Recently, Storelli (2008) noted that the cadmium level in edible marine species from the Adriatic Sea was highest in cephalopods (0.18–0.59 mg/kg dry weight), followed by the concentration in crustaceans (0.02–0.04 mg/kg w.w.) and in fish (0.01–0.05 mg/kg w.w.). A comparison of the cadmium concentrations in fish from different parts in the world is presented by Castro-Gonzalez and Mendez-Armenta (2008). The 1997 UK Total Diet Study (Ysart *et al.*, 2000) mentioned an average cadmium content of 0.013 mg/kg fresh weight for fish.

Celik and Oehlenschläger (2007) showed that, in Turkish supermarkets, fishery products from the region exhibited high cadmium contents (0.025 mg/kg w.w. in canned anchovy fillets, and 0.18 mg/kg in canned tuna).

TABLE 47
Cadmium concentrations as measured in different marine species

Species	Location	Cadmium Edible part (mg/kg wet weight)	Cadmium Hepatopancreas	Reference
<i>Illex coindetii</i>	Mediterranean Sea	0.13	2.48	Storelli, Barone & Marcotrigiano, 2005
<i>Octopus salutii</i>	Mediterranean Sea	0.77	9.65	Storelli, Barone & Marcotrigiano, 2005
<i>Eledone cirrhosa</i>	Mediterranean Sea	0.23	6.05	Storelli, Barone & Marcotrigiano, 2005
<i>Eledone moschata</i>	Mediterranean Sea	0.20	5.46	Storelli, Barone & Marcotrigiano, 2005
<i>Sepia elegans</i>	Mediterranean Sea	0.30	4.08	Storelli, Barone & Marcotrigiano, 2005
<i>Sepia orbignyana</i>	Mediterranean Sea	0.87	18.03	Storelli, Barone & Marcotrigiano, 2005
<i>Gadus morhua</i>	Northeast Atlantic	< 0.001		Julshamn <i>et al.</i> , 2004
<i>Reinhardtius hippoglossoides</i>	Northeast Atlantic	0.0012		Julshamn <i>et al.</i> , 2004
<i>Scomber scombrus</i>	Northeast Atlantic	0.003		Julshamn <i>et al.</i> , 2004
<i>Clupea harengus</i>	Northeast Atlantic	0.0025		Julshamn <i>et al.</i> , 2004
<i>Sebastes marinus</i>	Northeast Atlantic	< 0.001		Julshamn <i>et al.</i> , 2004
<i>Pollachius virens</i>	Northeast Atlantic	0.001		Julshamn <i>et al.</i> , 2004
<i>Sprattus sprattus</i>	Northeast Atlantic	0.023		Julshamn <i>et al.</i> , 2004
<i>Melanogrammus aeglefinus</i>	Northeast Atlantic	0.008		Celik, Cakli & Oehlenschläger, 2004
<i>Gadus morhua</i>	Northeast Atlantic	0.008		Celik, Cakli & Oehlenschläger, 2004
<i>Pollachius virens</i>	Northeast Atlantic	0.009		Celik, Cakli & Oehlenschläger, 2004
<i>Merluccius merluccius</i>	Northeast Atlantic	0.017		Celik, Cakli & Oehlenschläger, 2004
<i>Merlangius merlangus</i>	Northeast Atlantic	0.004		Celik, Cakli & Oehlenschläger, 2004
<i>Scomber scombrus</i>	Northeast Atlantic	0.023		Celik, Cakli & Oehlenschläger, 2004
<i>Trachurus trachurus</i>	Eastern Mediterranean Sea	0.027		Celik, Cakli & Oehlenschläger, 2004
<i>Sardina pilchardus</i>	Eastern Mediterranean Sea	0.020		Celik, Cakli & Oehlenschläger, 2004
<i>Engraulis encrasicolus</i>	Eastern Mediterranean Sea	0.058		Celik, Cakli & Oehlenschläger, 2004
<i>Dicentrarchus labrax</i>	Eastern Mediterranean Sea	0.046		Celik, Cakli & Oehlenschläger, 2004
<i>Mugil cephalus</i>	Western Africa	0.11 (dry weight)		Sidoumou <i>et al.</i> , 2005
<i>Argyrosomus regius</i>	Western Africa	0.005 (dry weight)		Sidoumou <i>et al.</i> , 2005
<i>Pegusa lascaris</i>	Western Africa	0.273 (dry weight)		Sidoumou <i>et al.</i> , 2005
<i>Pagrus auriga</i>	Western Africa	0.04 (dry weight)		Sidoumou <i>et al.</i> , 2005
<i>Buccinum undulatum</i>	France	1.7		Amiard <i>et al.</i> , 2008
<i>Chlamys nobilis</i>	China	4.2		Amiard <i>et al.</i> , 2008
Abalone	Australia	0.12		Fabris, Turoczy & Stagnitti, 2006
Snapper	Australia	0.02		Fabris, Turoczy & Stagnitti, 2006
Lobster	Australia	0.02		Fabris, Turoczy & Stagnitti, 2006
Black scabbardfish	Madeira	0.01		Alfonso <i>et al.</i> , 2007
Black scabbardfish	Azores	0.03		Alfonso <i>et al.</i> , 2007
Pacific cod	Aleutian Chain Alaska	0.009		Burger <i>et al.</i> 2007

3.3.3.6 Lead

Lead is one of the most ubiquitous metals known to humans, and it is detectable in practically all phases of the environment and in all biological systems. Environmental levels of lead have increased more than a thousandfold in the past three centuries as a result of human activity. The greatest increase occurred between the years 1950 and 2000 (Castro-Gonzalez and Mendez-Armenta, 2008). The situation with lead in fish is similar to that of cadmium. The lead concentration in the edible parts of fish is generally low. During the Norwegian monitoring programme in the Barents Sea, Norwegian Sea and North Sea, Julshamn *et al.* (2004) found lead concentration ranging from < 0.0005 to 0.01 mg/kg w.w.. Figures in the same range have also been published by Celik, Cakli and Oehlenschläger (2004) for Northeast Atlantic fish species (0.002–0.015 mg/kg w.w.). In the dietary study for the United Kingdom of Great Britain and Northern Ireland (above) an average lead content of fish of 0.02 mg/kg was reported. Higher concentrations of lead have been reported from areas with high industrial activity and from waters with no or little exchange with the world oceans (e.g. Baltic Sea).

3.3.3.7 Mercury

Mercury is an important pollutant and one of the most studied because it is very toxic and accumulates in organisms, particularly in fish. Mercury is released into the environment from both natural and anthropogenic sources. It is estimated that annual natural emissions from continental sources are approximately 1 000 tonnes. In pre-industrial times, input to the oceans is thought to have been about 600 tonnes. Today, however, this has increased to approximately 2 000 tonnes owing to the re-emission of mercury deposited as a result of human activities.⁶ Methylmercury rather than inorganic mercury is bioconcentrated because it is better retained by organisms at various levels in the food chain. The key factor determining the concentration of mercury in the biota is the methylmercury concentration in water, which is controlled by the relative efficiency of the methylation and demethylation processes. Anoxic waters and sediments are an important source of methylmercury, apparently as the result of the methylating activity of sulphate-reducing bacteria (Morel, Kreapiel and Amyot, 1998).

Mercury is of great interest to consumers, who are concerned as to whether it can cause neurological effects at low dose levels. The effects of organic mercury exposure at high levels have been demonstrated in several large-scale poisonings, particularly those in Japan and Iraq in the 1950s, 1960s and 1970s (for a review, see Rasmussen, Nettleton and Morrissey, 2005). These epidemics showed that organic mercury, in sufficient concentrations, is a potent neurotoxin that is especially harmful to the developing nervous system. As the most common form of human exposure to organic mercury is through seafood consumption, several epidemiological studies have examined the relationship between maternal fish intake and health effects in humans, especially in the foetus (Myers *et al.*, 2003; Myers, Davidson and Strain, 2007; Hibbeln *et al.*, 2007; Oken *et al.*, 2005; Spurgeon, 2006; Mergler *et al.*, 2007; Hightower and Moore, 2003; Khaniki *et al.*, 2005; Chen *et al.*, 2008; Choi and Grandjean, 2008; Choi *et al.*, 2008a; Levenson and Axelrad, 2006; Hughner, Maher and Childs, 2008; Koren and Bend, 2010; Oken *et al.*, 2008; Guldner *et al.*, 2007).

Recreational anglers who consume high amounts of fish are another group that can accumulate high amounts of mercury, e.g. in hair and blood (Lincoln *et al.*, 2011).

A protective effect of selenium against mercury toxicity has been demonstrated in animal models. As interactions between selenium and mercury and their molar ratios in seafood are essential factors in evaluating risks associated with dietary mercury

⁶ www.ec.gc.ca/mercure-mercury/default.asp?lang=En&n=A177A336-1

exposure, it has been argued that mercury content alone is inadequate (Kaneko and Ralston, 2007). However, there are also other reports that are less convincing about the role of selenium as a potential protective factor against mercury developmental neurotoxicity (Choi *et al.*, 2008b). Kaneko and Ralston (2007) found molar selenium/mercury ratios of 17.6 in striped marlin, 14.1 in yellowfin tuna, 13.1 in mahi mahi, 12.8 in skipjack tuna, 11.4 in spearfish, 10.8 in wahoo, 6.7 in sickle pomfret, 5.3 in albacore tuna, 5.2 in bigeye tuna, and 4.1 in blue marlin. In all fish investigated, the molar selenium/mercury ratio was higher than one, with the exception of mako shark, where it was 0.5. Plessi, Bertelli and Monzani (2001) found high molar selenium/mercury ratios in 25 marine fish species from saltwater and freshwater, ranging from 14.3 in hake to 1.25 in dogfish. In edible portions of 14 shellfish species, the molar selenium/mercury ratios were much higher, ranging from 4.2 in spiny spider crab to 33.3 in blue mussel.

Table 48 demonstrates some mercury and organic mercury concentrations in the edible parts (muscles) of seafood from different locations in the world based on Sahuquillo *et al.* (2007) (Spain) and Yamashita, Omura and Okazaki (2005) (Japan). The table shows that methylmercury is always a high proportion of total mercury, ranging up to 80 percent. The table also shows that predatory species show higher mercury contents compared with pelagic species and demersal species.

Bustamante *et al.* (2006) measured the total and organic mercury concentrations in cephalopods from Northeast Atlantic waters. High mercury concentrations were found in whole cephalopods ranging from 0.04 mg/kg to 3.56 mg/kg w.w.. The authors concluded that mercury is present in cephalopods mainly in the organic form in which the metal is likely to be bound to muscle proteins. Because organic mercury is highly bioavailable, cephalopods should be considered a significant source of mercury for consumers.

In edible parts (flesh) of the horned octopus (*Eledone moschata*), Storelli and Marcotrigiano (2004) reported a total mercury content of 0.36 mg/kg w.w., and Storelli *et al.* (2006) found total mercury concentrations in the flesh of six cephalopod species from the Mediterranean Sea ranging from 0.11 mg/kg w.w. in *Loligo vulgaris* to 0.87 mg/kg in *Octopus vulgaris*.

Knowles, Farrington and Kestin (2003) found the highest levels of total mercury in swordfish, marlin and shark (1.0–2.2 mg/kg fresh weight) in fish and shellfish imported into the United Kingdom of Great Britain and Northern Ireland and in farmed fish of the United Kingdom of Great Britain and Northern Ireland. All other samples of captured fish and the farmed salmon and trout of the United Kingdom of Great Britain and Northern Ireland were found to be below the legal limits in Europe (0.5 mg/kg w.w.). Julshamn *et al.* (2006) demonstrated that low levels of mercury were detected in fillets of Greenland halibut from the Barents Sea, with an average content of 0.15 mg/kg w.w. in 29 specimens and 0.39 mg/kg w.w. in 40 specimens.

The highest mercury concentrations have been reported for red-meat products from cetaceans in the Republic of Korea (Endo *et al.*, 2007) and on the Japanese market (Endo *et al.*, 2004). The total mercury concentrations in red-meat products in the Republic of Korea were highest in the false killer whale (9.66 mg/kg w.w.), bottlenose dolphin (10.6 mg/kg w.w.), and killer whale (13.3 mg/kg w.w.). In Japan, the levels of total mercury and methylmercury in toothed whale red meat, the most popular whale product, were 8.94 mg/kg w.w. and 5.44 mg/kg w.w., respectively. The total mercury concentrations in the boiled liver have been found to be high enough (388 mg/kg w.w.) to cause acute intoxication even from a single ingestion.

An in-depth discussion about seafood intake, contaminants and human health can be found in the following reviews: Mozaffarian and Rimm, 2006; Mozaffarian, 2009; Genius, 2008; Budtz-Jørgensen, Grandjean and Weihe, 2007; Smith and Sahyoun, 2005; Marti-Cid *et al.*, 2007.

TABLE 48

Mercury and methylmercury concentrations in edible part of some fish species

Location	Species	Total mercury (mg/kg wet weight)	Methyl mercury
Spain	<i>Thunnus thynnus</i>		0.6
Spain	<i>Scomber scombrus</i>		0.064
Spain	<i>Xiphias gladius</i>		0.48
Spain	<i>Solea vulgaris</i>		0.028
Spain	<i>Mytilus edulis</i>		Below detection limit
Spain	<i>Merluccius merluccius</i>		0.14
Spain	<i>Mora moro</i>		0.2
Japan	<i>Beryx splendens</i>	0.78	0.52
Atlantic Ocean	<i>Thunnus thynnus</i>	0.42	0.29
Pacific Ocean	<i>Thunnus thynnus</i>	0.59	0.49
Pacific Ocean	<i>Thunnus obesus</i>	0.98	0.69
Atlantic Ocean	<i>Xiphias gladius</i>	0.47	0.34
Indian Ocean	<i>Thunnus maccoyii</i>	0.27	0.19
Japan	<i>Trachurus japonicus</i>	0.02	0.02
Mediterranean Sea	<i>Thunnus alalunga</i>	0.88	

In order to reduce the content of mercury in predatory fish such as tuna for the market, first experiments have been conducted to look for the possibility of decreasing the mercury content in bluefin tuna (*Thunnus orientalis*) held in aquaculture by dietary modification (selecting dietary fish species according to their mercury content) (Nakao *et al.*, 2007).

Kraepiel *et al.* (2003) analysed the sources and variations of mercury content in yellowfin tuna off Hawaii. Based on the fact that mercury concentration in this fish species has not increased in the last 30 years, they hypothesized that methylmercury is formed in the deep seas or in sediments, where mercury concentrations have been little affected by human activities.

Recently, the Zero Mercury Working Group and the Mercury Policy Project, both non-governmental organizations (NGOs), have published the report *Mercury in fish – A global health hazard* (Anon., 2009). In this report, some new data on mercury in fish (especially predatory fish known as mercury accumulators owing to their long life span and the fact that they are at the end of the marine food web) from India, the Philippines and Europe are presented. In addition, information is provided about methylmercury in marine mammals and the implication for arctic populations. There are also chapters about consumer exposure and health risks. The report ends with general and specific recommendations for particular countries and populations.

3.3.3.8 Seaweed (algae)

About 15.8 million tonnes of aquatic plants (FAO, 2010b) were cultivated in 2008. These aquatic plants are used predominantly in Asian countries, mostly for direct consumption and in Western countries for the extraction of agar, carrageenans and alginates used as food ingredients. However, in European countries direct consumption of algae is steadily growing (e.g. as a part of sushi).

Algae accumulate heavy metals and have been used as biomonitors for metal pollution and to evaluate the quality of the environment. Green algae have a lower metal-binding capacity than brown algae. Limits for edible seaweed exist only in French legislation: lead < 0.1 mg/kg dry weight, cadmium < 0.5 mg/kg dry weight, mercury < 0.1 mg/kg dry weight, and inorganic arsenic < 3 mg/kg dry weight. Results of algae samples from the Atlantic and the Pacific Ocean obtained from specialist shops throughout Spain (Besada *et al.*, 2009) showed that the cadmium concentration of most algae species investigated exceeded 0.5 mg/kg dry weight. Concerning lead, several samples had concentrations below the detection limit (< 0.008 mg/kg), and the

maximum values found were about 1 mg/kg. Values for mercury were low in all algae species (0.001–0.057 mg/kg dry weight). The authors found that metal concentrations for the algae from the two production areas (Atlantic and Pacific) analysed in the study were very similar and could not be differentiated by multivariate analysis.

3.3.3.9 Bioaccessibility

Amiard *et al.* (2008) make a statement that for elements that are considered the most toxic, e.g. cadmium, bioaccessible concentrations are generally consistently lower than total concentrations. Therefore, it may be relevant to take this into account for a more accurate assessment of seafood quality in order to meet the needs of both human health security and the economic interests of the fishery industry, e.g. shellfish farmers. They also note that the way of cooking also influences metal bioaccessibility and, thus, recommend that bioaccessibility is considered when estimating the dietary intake of metals by human consumers.

The bioaccessibility of mercury and methylmercury in swordfish has been determined by Torres-Escribano, Vélez and Montoro (2010). Bioaccessible mercury concentrations were 38–83 percent (average 64 percent \pm 14 percent) of total mercury.

3.3.4 Emerging hazards – allergies (Andrea Lopata)

3.3.4.1 Allergy and adverse reactions to seafood – an overview

Seafood plays an important role in human nutrition and health. The growing international trade in seafood species and products has added to the popularity and frequency of consumption of a variety of seafood products across many countries. However, increased production and consumption of seafood has resulted in more frequent reports of health problems among consumers as well as processors of seafood.

Adverse reactions to seafood can generate reactions mediated by the immune system (allergies) as well as non-immunological reactions (Lehrer, Ayuso and Reese, 2003; Lopata and Potter, 2000). These reactions can result from exposure to the seafood itself or various non-seafood components in the product. Non-immunological reactions to seafood can be triggered by contaminants such as parasites, bacteria, viruses, marine toxins and biogenic amines. Biogenic amines are mostly found in “spoiled” fish (scombroid poisoning) whereas marine biotoxins, generated by algae, can be detected in certain fish (ciguatera toxin) as well as in filter feeders such as mussels. Ingredients added during the processing and canning of seafood can also cause adverse reactions (see below). Importantly, all these substances can trigger symptoms that are similar to true allergic reactions, which are mediated by antibodies produced by the immune system against specific allergens (Table 49).

Because of the similarity in clinical reactions of affected consumers and workers, it is of fundamental importance to differentiate adverse reactions from true seafood allergies and understand the underlying mechanisms of allergic reactions and molecular nature of these allergens. The implicated allergens, epidemiology and prevalence are discussed below.

TABLE 49
Adverse reactions to seafood produced by various substances

Aetiology	Seafood implicated	Clinical symptoms	Time of symptom onset
Bacterial <i>Salmonella</i> , <i>Vibrio</i> , <i>Aeromonas</i> , <i>Listeria</i> , etc.	Fish, crustaceans, molluscs		
Viral Hepatitis A, rota-, astrovirus, small round viruses, etc.	Crustaceans Molluscs	Dermatological	
Parasites Anisakis Diphyllobothrium, etc.	All fish and cephalopods (e.g. squid)	Gastrointestinal	From a few minutes to several hours
Toxins Scombrototoxin Ciguatera toxin Algal toxins	Scombroid fish Reef fish All mollusc species	Neurological Respiratory	
Allergens	Fish Crustaceans Molluscs		

3.3.4.2 Classification of seafood groups

Patients with an allergy to seafood may fail to identify the offending seafood species, often as a result of confusion regarding the diversity of seafood consumed and the different common names used to describe seafood. The three most important seafood groupings include the arthropods, molluscs and fish. The two invertebrate phyla of arthropods and molluscs are generally referred to as “shellfish” in the context of seafood consumption (Table 50). Most seafood species are edible, and more exotic ones, such as sea cucumber, jellyfish and sea urchins, are consumed in small amounts around the world.

TABLE 50
Classification of seafood groups causing allergies, representative species, common symptoms experienced and main allergens implicated

Group	Class	Common Name	Allergens	Molecular weight (kDa)
Arthropoda	Crustaceans	Crab, rock lobster, prawn, shrimp, krill, barnacle	Tropomyosin Arginine kinase Myosin light chain	34–39 40 20
Molluscs	Gastropods	Abalone, snail, whelk	Tropomyosin	34–39
	Bivalves	Clam, oyster, mussel, cockle	Tropomyosin	34–39
	Cephalopods	Squid (cuttlefish), octopus	Tropomyosin	34–39
Fish	Bony fish	Salmon, hake, tuna, herring, sardine, mackerel, carp	Parvalbumin Collagen Vitellogenin Roe Anisakis parasite	12 110–210 205 9–154
	Cartilaginous fish	Sharks, rays	?	37–50

Note: Allergens indicated with “?” are not well characterized.

Crustaceans are classified as arthropods together with spiders and insects. More than 30 000 living crustacean species are found worldwide, and a large number of varieties are consumed raw or cooked. The Mollusca is a large and diverse group, subdivided into the classes bivalves, gastropods and cephalopods (Table 50). It comprises more than 100 000 different species, including several economically important seafood groups such as mussels, oysters, abalones, snails and squid.

The last of the seafood groups are the fish, which can be divided into bony fish and cartilaginous fish. Most edible fish belong to the bony fish, whereas sharks and rays are cartilaginous and belong to a different order (Table 50). Most studies on fish allergens have focused on cod and carp. Although there are more than 20 000 different species of fish, consumption depends on regional availability.

3.3.4.3 *Disease and epidemiology*

Allergic reactions to seafood are generated by otherwise harmless proteins that react as allergens in very few individuals. The immune system of sensitized individuals produces specific antibodies, which are responsible for the allergic reaction. Symptoms range from mild urticaria and oral allergy syndrome to life-threatening anaphylactic reactions (Lopata and Potter, 2000; Sicherer, Munoz-Furlong and Sampson, 2004; Wild and Lehrer, 2005). The pattern of allergic symptoms after ingestion of seafood appears similar to the symptoms experienced with other foods. Reactions are immediate, reported mostly within 2 h; however, late-phase reactions have been reported up to 8 h after ingestion, particularly in relation to snow crab, cuttlefish, limpet and abalone (Lopata, Zinn and Potter, 1997; Villacis *et al.*, 2006). Patients may have a single symptom but there is often a multiorgan involvement. Importantly, respiratory and/or anaphylactic reactions are often seen after ingestion of allergenic seafood. The “oral allergy syndrome” seems to be very often experienced by crustacean-allergic subjects. Symptoms occur within minutes of ingestion of crustaceans and include itching and angioedema of the lips, mouth and pharynx. Shrimp has also been implicated in food-dependent exercise-induced anaphylaxis. It seems that atopic individuals are at greater risk of developing anaphylactic reactions.

The appearance of allergic symptoms results not only from ingestion of seafood but can also be triggered by inhaling cooking vapours and handling seafood in the domestic as well as in the working environment (see below) (Goetz and Whisman, 2000; Jeebhay *et al.*, 2001; Taylor *et al.*, 2000). Symptoms manifest mainly as upper- and lower-airway respiratory symptoms and dermatitis, while anaphylaxis is rarely seen with this type of exposure.

Importantly, there are a number of individuals who have reacted to seafood and wish to continue to eat seafood. Therefore, it is crucial to establish that any adverse reaction was indeed IgE-mediated (allergic) and correctly identify the specific seafood species implicated. While a detailed history is essential, the identification of the implicated seafood species, using specific diagnostic procedures, is of importance, particularly if the seafood product is not properly identified. Sensitized individuals need to be advised about the potential dangerous consequences of continued exposure.

Diagnostic methods of establishing a true seafood allergy include skin-prick testing and the quantification of specific IgE antibodies using assays such as the ImmunoCAP or allergen-microarray. However, positive test results do not necessarily confirm clinical sensitivity, nor do negative results exclude possible clinical reactivity. Moreover, possible cross-reactivity between tropomyosin from crustaceans and molluscs with tropomyosin from insects and mites may be important for some individuals.

Several studies have attempted to establish how much seafood is needed to trigger an allergic reaction by challenging the individuals with the offending food. So-called double-blind controlled food challenges (DBPCFCs) have indicated that as little as 32 mg of shrimp-protein extract, about four medium-sized shrimps, caused allergic reactions.

3.3.4.4 Prevalence

The prevalence of seafood allergy is usually higher when seafood consumption plays a greater part in the diet of the observed community. It is generally considered that crustaceans and fish are among the four foods that most commonly cause severe food anaphylaxis. It is estimated that about 30 000 food-induced anaphylactic events are seen annually in the United States of America alone, of which about 200 are fatal. A recent study established that seafood allergies are a significant health concern – affecting approximately 6.5 million people in the United States of America (more than twice as common as peanut allergy). From a telephone survey of 14 948 individuals, 2 percent reported a shellfish allergy that was almost five times more common among adults compared with children (Sicherer, Munoz-Furlong and Sampson, 2004) (Table 51). Of the subjects with allergies to crustaceans and molluscs, 38 percent and 49 percent, respectively, reported reactions to multiple species, and 14 percent reacted to both shellfish groups, suggesting less cross-reactivity between crustaceans and molluscs. In a study conducted in France among 580 patients with adverse reactions to food, 34 percent demonstrated specific IgE to crab. A study from Spain among 355 children established that 6.8 percent of patients reacted to crustaceans by skin-prick testing. A study from South Africa on 105 individuals with perceived adverse reactions to seafood confirmed sensitization to prawns and rock lobster in 47 percent and 44 percent, respectively. Of the 131 positive reactions by ImmunoCAP, 50 percent reacted to four different crustacean species.

Seafood allergy is common in Western countries such as the United States of America, Europe and Australia. However, also in Asian countries, allergic reactions to seafood, particularly shellfish, are significant among children and adults (Table 51) (Hill *et al.*, 1997). Moreover, more seafood is readily available to a wider range of populations and countries owing to improved transportation, shipping and the general globalization of food supply, as well as increasing socio-economic standards in regions such as Southern Europe. The likelihood of becoming sensitized to a particular food allergen seems to correlate with geographical eating habits, so a seafood allergy to a particular seafood species is more prevalent in countries where this seafood is part of the stable diet.

TABLE 51

Prevalence of sensitization to shellfish in various countries among individuals (adults and children) with food allergy

Country	Number of individuals investigated	Prevalence (%)	
		Shellfish	Fish
Thailand	202	22	ND
Philippines	38	58	63
Singapore	334	15	ND
Singapore	227	39	ND
Taiwan Province of China	392	21	ND
Indonesia	600	24	18
China, Hong Kong SAR	80	ND	17.5
Japan	97	ND	9.3
France	580	34	ND
Spain	355	6.8	17.8
South Africa	105	55	20
Australia*	620	ND	0.07
United States of America*	14 948	2	0.4

Notes: A survey among the general population is indicated by an asterisk. Sensitization established by skin-prick-testing and/or quantification of specific IgE antibody to shellfish and fish. ND = Not determined.

3.3.4.5 Allergens in seafood

Fish: The most comprehensive study on a seafood allergen was the analysis of the allergen from codfish, Gad c 1 (originally named “Allergen M”), conducted in the early 1970s (Elsayed and Bennich, 1975).. Gad c 1 belongs to a group of muscle tissue proteins known as parvalbumins (Table 50). These control the flow of Ca^{2+} in and out of cells and are only found in the muscles of amphibians and fish (Van Do *et al.*, 2005). The molecular weight of this protein can vary between 10 and 13 kDa in different fish species and is divided into two distinct phylogenetic lineages. These include the α -isoform with an isoelectric point above pH 5 and a β -isoform with an isoelectric point below pH 5. The majority of allergenic parvalbumin sequences that have been deduced belong to the β lineage. This phenomenon is attributed to conserved structural features and amino-acid similarities of parvalbumin among fish species. The 12 kDa allergen from cod shares about 60–80 percent amino-acid homology with similar proteins from hake, carp, pike and whiting, and may explain some of the cross-reactivity in fish-allergic patients. While parvalbumin is the main allergen identified in most studies, it displays variable IgE cross-reactivity, reflected in differential clinical reactivity, where some patients can consume one but not the other fish species.

However, additional allergens have been identified, such as collagen (from the skin and tissue), as well as the hormone vitellogenin, found particularly in fish roe (caviar) (Table 50).

In addition to these allergens derived from the fish themselves, contaminants such as the parasite *Anisakis* can cause allergic reactions (Audicana and Kennedy, 2008). The eight allergens characterized are tropomyosin, cross-reacting to shellfish allergens, as well as paramyosin and protease inhibitors. A recent study has demonstrated that these parasites can also cause allergic sensitization among fish processing workers (Nieuwenhuizen *et al.*, 2006).

Shellfish: The major allergens responsible for ingestion-related allergic reactions due to crustaceans are tropomyosins, while molluscs seem to contain, in addition to tropomyosin, other less well-characterized allergens (Table 50). It is noteworthy that crustacean and mollusc allergens do not cross-react with fish allergens, as these are mostly parvalbumins.

In the early 1980s, Hoffman and coworkers identified a heat-stable IgE-antibody-binding allergen in shrimps, which was later demonstrated to be tropomyosin. Shrimp tropomyosin has a slightly acidic isoelectric point and seems to have minor glycan modifications and lacks cysteine residues. Tropomyosin is a water-soluble and heat-stable protein with molecular weights ranging from 34 to 39 kDa (Reese, Ayuso and Lehrer, 1999). While tropomyosin migrates in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as a single band, the protein is, in its native state, a coiled-coil homodimer with a much higher molecular weight. Tropomyosin has a highly conserved amino-acid sequence among different invertebrate organisms and is present in muscle as well as in non-muscle cells. It is present in all eukaryotic cells, where they are associated with the thin filament in muscle, and microfilaments in many non-muscle cells. Tropomyosin, together with actin and myosin, plays a role in the contractile activities and morphology of these cells. IgE-binding studies with various species and sensitized individuals have demonstrated a variety of tropomyosin epitopes. This suggests the existence of species-specific epitopes (in addition to common epitopes) in crustaceans, but also in other invertebrates, such as molluscs and insects.

Furthermore, it has been shown that there are three different isoforms of tropomyosin relating to different functional needs (fast, slow-twitch and slow-tonic), identified by amino-acid sequence analysis. The fast isoform is mostly found in the abdominal muscle (tail), while the slow isoform is mainly associated with muscle

obtained from the legs. However, both forms can be found in abdominal and leg muscle, with amino-acid homology of up to 100 percent.

In addition to tropomyosin, other allergens have been identified and characterized in crustaceans such as arginine kinase. A number of proteins with molecular masses ranging from 8 kDa to 89 kDa that bind serum IgE antibodies of allergic individuals have also been demonstrated, although not immunochemically identified.

Importantly, tropomyosin is not only a crustacean allergen. It has been confirmed in a number of mollusc species (Taylor, 2008). Mollusc allergens have not been as well studied as those of fish or crustaceans. However, it has become apparent that molluscs, such as mussel, oyster, squid, limpet and abalone, are significant food allergens to exposed populations. However, in addition, molluscs contain other non-tropomyosin allergens such as heavy-chain myosin, haemocyanin and amylase (Taylor, 2008). As mentioned above, arginine kinase in molluscs also seems to be allergenic, accounting for an additional degree of cross-reactivity among these two seafood groups.

3.3.4.6 *Detection and control*

The labelling of foods containing material derived from fish and crustaceans has already become mandatory in some countries such as in the United States of America, Europe, and Japan. For labelling purposes, fish and crustaceans have been recognized as important food allergens for some time. However, only recently has the European Union (Member Organization) adapted its guidelines to include molluscs as a separate food allergen, based on the limited cross-reactivity to crustacean allergens.⁷ A recent comparative study of two newly developed ELISA systems has demonstrated high sensitivity (1 µg/g food) as well as reasonable recovery and reproducibility rates (Sakai *et al.*, 2008). Nevertheless, a certain degree of cross-reactivity to cockroach and mollusc tropomyosin has also been noted. This cross-reactivity might be of greater importance considering the large variety of tropomyosins identified in crustaceans and also molluscs (see below). Moreover, the detection of processed crustacean, rather than raw crustacean, is dependent on the recognition of tropomyosin not only in the monomeric form but also possible oligomers and fragments of tropomyosin, which might still have allergenic activity.

Additional problems might arise not only with the different processed crustacean and mollusc species but also with the increasing number of food, medical and health products derived from shellfish. Chitin and chitosan are among the emerging materials that are being developed and applied widely in the food, biotechnology, pharmaceutical and medical fields. The main obstacle for the future use of chitosan is the residual amount of about 1 percent protein in industrially produced chitosan. Allergic reactions after consuming chitosan-containing food have been reported. However, the contribution of the thermostable tropomyosin or other yet unidentified crustacean allergens has not been demonstrated. Other pharmaceutical products derived from crustaceans are glucosamine, a natural aminomonosaccharide, which is frequently used as a therapeutic supplement for joint inflammation. It was previously indicated as a potential risk for shellfish-sensitive individuals because it was derived from shellfish chitosan. However, it has been demonstrated not to be allergenic using DBPCFC in 15 shrimp-allergic patients. In addition, food products can also unexpectedly be derived from crustaceans. Surimi (seafood paste) is usually produced from fish, but in some countries it can contain a variety of crustacean species.

⁷ Opinion of the EFSA Scientific Panel available at: www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178623594074.htm

3.3.4.7 Processing and changes in allergenicity

Food is subjected to a large variety of processing conditions to prolong storage or improve sensory qualities. Many different processes are used, often in combination, but they can be generally categorized into thermal and non-thermal procedures. A workshop evaluated the effects of food processing on the allergenicity of food allergens (Thomas *et al.*, 2007). Various food processes have been implemented to reduce the allergenicity of certain foods, but few studies have focused on seafood.

As invertebrate tropomyosin is typically a lysine-rich protein (up to 12 percent in scallops) it reacts easily with reducing sugars through the Maillard reaction during food processing such as grilling, steaming and roasting. The brown colour of dried seafood is caused by the Maillard reaction. Studies on the effect of sugar residues on two different mollusc species showed opposite effects. Heating of scallops (a bivalve) in the presence of sugar residues increased IgE binding, as demonstrated by competitive ELISA, while a decrease in allergenicity was observed for squid (calamari) in the presence of the reducing sugar ribose (Nakamura *et al.*, 2006). The interpretations of these contradicting results are difficult as IgE-binding activity is not always correlated with clinical reactivity.

Non-thermal processes have been also investigated, such as gamma radiation of crustaceans and molluscs, which resulted in reduced IgE-binding capacity of the allergens as well as high-intensity ultrasound treatment of shrimp (Table 52). Meyer-Pittroff, Behrendt and Ring (2007) suggest that pressure of more than 600 MPa causes reversible and irreversible changes to the secondary, quaternary and tertiary structure, particularly in helical proteins, and they demonstrated reduced allergenicity. Nevertheless, complete loss of allergenicity or allergen concentrations has not been demonstrated, which is probably due to the fact that even small protein fragments of about 3.5 kDa can still cross-link mast cell IgE and elicit an allergic reaction (Thomas *et al.*, 2007). Moreover, the solubility and extractability of treated tropomyosin might be affected and result in underdetection, as has been demonstrated for radiation-treated crustaceans and molluscs. In addition, it cannot be ruled out that processing has different effects on the less well-characterized seafood allergens. Most of the processes investigated to reduce allergenicity are purely experimental, but this is an important area of research into seafood allergy that should be further explored. Furthermore, the challenge of maintaining the flavour and texture of seafood during these processes will be of importance.

The allergenicity of seafood allergens also seems to vary with storage procedures. Codfish stored for several days (at 4 °C) displayed a much higher IgE reactivity than very fresh fish. These biochemical changes of allergens, even during longer freezing periods, may be attributed to the natural development of components such as formaldehyde in fish tissue, which might affect the allergenicity of some proteins.

3.3.4.8 Occupational allergy to seafood

Disease, epidemiology and prevalence: The fishing and fish processing industry has experienced tremendous growth in recent years. FAO (2012) estimated that the number of people engaged in fishing, aquaculture and related activities worldwide increased from 13 million in 1977 to about 55 million in 2010. Among these workers, Of these, an estimated 7 million people were occasional fishers and fish farmers (of whom 2.5 million in India, 1.4 million in China, 0.9 million in Myanmar, and 0.4 million each in Bangladesh and Indonesia). More than 87 percent of all people employed in the fisheries sector in 2010 were in Asia, followed by Africa (more than 7 percent), and Latin America and the Caribbean (3.6 percent). Increased levels of production and processing of seafood continue to lead to more frequent reporting of occupational health problems such as asthma and other allergic reactions (Jeebhay *et al.*, 2001). These

occupational health problems result in increased incapacity and absenteeism among affected workers.

TABLE 52
Processing techniques evaluated to reduce allergenicity in seafood

Species	Type of processing	Type of analysis	Results
Shrimp (<i>Penaeus vannamei</i>)	Gamma radiation + heat	SDS-PAGE* + Immunoblotting	Decrease in allergenicity with increased dose
Shrimp (<i>Penaeus vannamei</i>)	Gamma radiation	Immunoblotting + competitive ELISA*	Increased allergenicity at (< 10 kGy), but decreased at (> 10 kGy)
Shrimp (<i>Penaeus vannamei</i>)	High-intensity ultrasound	Immunoblotting + competitive ELISA	Decrease in allergenicity
Brown shrimp (<i>Penaeus aztecus</i>)	Gamma radiation	SDS-PAGE + competitive ELISA	Decrease in allergenicity
Tuna (albacore and yellowfin)	Heat treatment	SDS-PAGE, immunoblot and histamine release assay	Mixed results (i.e. some patients reacted and others did not)
Tuna and salmon	Heat treatment	SDS-PAGE and immunoblot	Strong decrease in IgE-binding to allergens; however, <i>in vitro</i> tests often did not correlate with the clinical relevance of fish hypersensitivity

* ELISA = enzyme-linked immunosorbent assay; SDS-PAGE = sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Workers in the fishing and seafood processing industries are commonly exposed to seafood, especially those involved in either manual or automated processing of crabs, prawns, mussels, fish and fishmeal. Other workers associated with potential high-risk exposure to seafood include: oyster shuckers, laboratory technicians and researchers, jewellery polishers, restaurant chefs and waiters, fishmongers and fishers. Occupational seafood allergy was documented for the first time in 1937, when a fisher was reported to have developed asthma, angioedema and conjunctivitis after handling codfish. Since then, coinciding with the significant growth in the seafood industry, seafood allergy symptoms ranging from rhinitis to conjunctivitis, asthma, urticaria, protein-contact dermatitis and occasional systemic anaphylactic reactions have been reported in seafood processing workers. The respiratory tract is often the primary route of occupational exposure as a result of inhalation of aerosols generated during seafood processing. However, reactions can also occur via the dermal route as a result of direct handling of the seafood itself.

The prevalence of occupational asthma in seafood processing workers is estimated to be between 2 and 36 percent, and that of occupational protein-contact dermatitis is 3–11 percent (Jeebhay *et al.*, 2001). From the limited scientific data available for all seafood groups, it seems that crustaceans produce a particularly strong allergic response in the workplace with sensitization rates of up to 26 percent (skin-prick testing) for king crab, rock crab and snow crab (Hefle *et al.*, 1995). The differences in prevalence observed can be due to differential exposure to seafood constituents and the allergenic potentials of the seafood proteins involved.

3.3.4.9 Occupational seafood allergens and exposure

Allergic reactions to seafood in the workplace are the result of exposure to seafood itself or to various non-seafood components present in the product. The aerosols generated by snow-crab and king-crab processing have been found to contain not only allergenic muscle proteins, but also crab exoskeleton, gills, kanimiso (internal organs) as well as background material such as sodium chloride crystals, cellulose, synthetic fibres, silicate, pigment constituent particles, and inorganic particles (silicon, aluminium and iron) (Desjardins *et al.*, 1995). Most of the airborne particles are irregular, and at least 30 percent are within the respirable range (< 5 µm), which can reach the deeper areas

of the lung. Environmental monitoring of seafood processing plants has also identified contaminated processing water (*Klebsiella pneumoniae* and *Pseudomonas*) as well as elevated levels of endotoxin ($> 50 \text{ EU/m}^3$) thought to be responsible for respiratory symptoms.

The constituents of fish juice, often associated with skin symptoms, comprise: traces of biogenic amines, histamine, and cadaverine; degradation compounds associated with post-mortem changes; digestive enzymes such as pepsin and trypsin; and proteins. Thus, storage conditions can influence the allergenic nature of seafood. In addition, biochemical sensitizers such as garlic, spices and preservatives added to seafood can also cause delayed allergic-contact dermatitis and general sensitization.

Limited evidence from dose-response relation studies indicates that the development of symptoms is related to the duration and intensity of exposure (Jeebhay *et al.*, 2005; Lopata *et al.*, 2005).

3.3.4.10 Prevention and control

Seafood processing plants vary in technology levels and processing procedures, with some smaller workplaces relying entirely on manual handling of seafood, and larger companies using highly automated processes. Common processing techniques and sources of potential high-risk exposure to seafood products are outlined in Table 53.

The lack of standardized methods to collect environmental samples and conduct analyses makes comparisons between various studies difficult. It is notable that, generally, much higher allergen concentrations have been obtained using personal sampling compared with area sampling. There is great variability of exposure within and among various jobs involved in seafood processing, with reported allergen concentrations ranging from 2 ng/m^3 in a fish market to $1\,000 \text{ ng/m}^3$ in a salmon processing plant (Table 54). Aerosolization of seafood components during processing has been identified as a potentially high-risk activity for sensitization through the respiratory route. The processes with high potential for aerosol exposure include: butchering/grinding, degilling, cracking and boiling of crabs; tailing of lobsters; “blowing” of prawns; scrubbing of shellfish; degutting, heading and cooking/boiling of fish; mincing of seafood; and cleaning of the processing line and storage tanks with high-pressure water.

Despite high levels of automation in larger workplaces, inadequate and poorly designed exhaust ventilation systems can pose high risks for workers. In addition, processes that generate dry aerosols, such as prawn blowing using compressed air, appear to generate higher particulate levels than wet processes using water jets.

Preventive measures are key to minimizing exposure to occupational diseases. Control measures that reduce the emission of bioaerosols in seafood processing plants include process separation or enclosure and the use of local extraction ventilation systems for processes and equipment. These changes can reduce aerosol concentrations by more than 100 times and prevent new asthma cases. Exposure monitoring for bioaerosols can evaluate the effectiveness of control measures in decreasing the risk of infection and allergic sensitization. In the case of skin exposure, the application of hand moisturizers in combination with appropriate cotton-lined gloves and plastic sleeves can protect workers.

TABLE 53
Causative agents and possible health effects

Causative agents	Health effects
Seafood proteins (muscle, blood, enzymes)	Rhinoconjunctivitis, asthma, urticaria, dermatitis
Seafood toxins	Toxic reactions
Vegetable dust additives (garlic, onion, spices)	Rhinoconjunctivitis, asthma, urticaria, dermatitis
Parasites (<i>Anisakis</i>)	Infection, rhinoconjunctivitis, asthma, urticaria
Micro-organisms (<i>Vibrio</i> , Hepatitis A)	Wound infection, sepsis
Bacterial toxins (endotoxin, histamine)	Organic dust toxic syndrome, mucous membrane irritation, rhinoconjunctivitis, asthma, urticaria
Mould in humid environments	Infection, rhinoconjunctivitis, asthma, urticaria, hypersensitivity pneumonitis

TABLE 54
Common processing techniques, allergen concentrations and asthma prevalence determined by high-risk exposure to seafood products

Seafood category	Processing techniques	Allergen concentrations (µg/m³)	Asthma prevalence	Source of potential exposure
Crustaceans Crabs, lobsters,	Cooking (boiling or steaming); tailing lobsters, cracking butchering and degilling crabs; cutting, grinding mincing, washing, cooling, heading, peeling; deveining; manual picking of meat; "blowing" shrimp (water jets or compressed air)	0.003–0.115	2–36	Inhalation of wet aerosols, dermal contact from unprotected handling of shrimp immersion in water containing
Molluscs Oysters, mussels	Washing, oyster shucking, shellfish, chopping, dicing, slicing	–	1–23	Inhalation of wet aerosols, dermal contact from unprotected handling
Finfish Various species	Heading, degutting, skinning, mincing, filleting, trimming	0.002–1.00	2–8	Inhalation of wet aerosols, inhalation of dry aerosols from fishmeal bagging and milling, dermal contact from unprotected handling

3.4 PHYSICAL HAZARDS (JÖRG OEHELENSCHLÄGER)

The physical hazards and physical defects include foreign objects that are capable of injuring the consumer and which are not normally found in aquatic products. These originate primarily from processing machinery, packaging or transportation/storage, but also objects that are intrinsic to the fish such as bones or shell fragments in bivalve molluscs. Another class includes aesthetically unpleasant but non-hazardous objects such as sand, insect fragments, filth and hair. It is not always simple to differentiate between the two classes of hazards.

The adverse health effects of physical hazards may be choking and injury, including laceration and perforation of tissues in the mouth, throat, stomach and intestines. Broken teeth and damage to gums may also result.

Although physical hazards rarely cause serious injury, they are among the most commonly reported cause of consumer complaints, because the injury occurs immediately or soon after eating and the source of the hazard is often easy to identify.

Many physical defects or physical hazards are mentioned and described in detail for a number of fishery products (fish-, crustacean- and mollusc-based) in the respective chapters of the first edition of the Code of Practice for Fish and Fishery Products, edited by FAO/WHO in 2009 (CAC, 2009c) in three languages (English, French, and Spanish).

Table 55 lists examples for physical hazards that are capable of injuring the consumer, the kind of injury and possible sources for the hazard.

TABLE 55
Physical hazards that may occur in seafood products

Hazard	Injury	Possible source
Hook	Trauma	Raw material
Machinery part	Dental	Processing
Jewellery, button, coin	Dental	Personal effects
Bone	Trauma	Raw material, processing
Wood splinter	Trauma	Processing, packaging material (boxes)
Glass	Trauma	Processing, packaging material (jars)
Hard plastic	Trauma	Processing, packaging material, personal effects
Hard shell fragments	Trauma/dental	Raw materials (molluscs, crustaceans)
Stone	Dental	Raw materials (mussels)

3.4.1 Bones

A hazard that is intrinsic to all fishery products is bones. Because bones are a natural part of the fish skeleton, any improper and/or insufficient removal of bones, especially in products designated as “practically boneless”, leads to complaints by consumers. Only bones of certain dimensions in length and width can be found by mouth feel, but if chewed or swallowed can be a hazard and cause lesions. Bones exceeding these dimensions are called defect bones.

In some of the fish standards of the CAC, a definition of a defect bone is given:

A bone in a package designated as boneless is a defect if it is “greater or equal to 10 mm in length, or greater or equal to 1 mm in diameter; a bone less than or equal to 5 mm in length, is not considered a defect if its diameter is not more than 2 mm. The foot of a bone (where it has been attached to the vertebra) shall be disregarded if its width is less than or equal to 2 mm, or if it can easily be stripped off with a fingernail” (CAC, 1995).

3.4.2 Metal inclusion

The FDA (2011f) has described metal inclusion as one of the two most common physical hazards. Metal fragments can cause injury to the consumer. Metal-to-metal contact, especially in mechanical cutting or blending operations, other equipment with metal parts that can break loose (such as moving wire mesh belts, injection needles, screens, portion control equipment, metal ties, sawing devices and can openers) are the most likely sources of metal that may enter food during processing.

3.4.3 Glass inclusion

The FDA (2011g) has described glass inclusion as the other of the two most common physical hazards. Glass fragments can cause injury to the consumer. Glass inclusion can occur whenever processing involves the use of glass containers. Normal handling and packaging methods, especially mechanized methods, can result in breakage. Most products packed in glass containers are intended as a RTE commodity.

3.4.4 Struvite crystals

Canned shrimp, tuna, salmon and other seafood products are sometimes found to contain small fragments of a substance that, at first glance, resembles glass.

These crystals are harmless and are known as “struvite” crystals or, chemically, as magnesium ammonium phosphate hexahydrate. They are formed from natural, normal constituents of the flesh of all seafoods after they are sterilized in the can.

While magnesium ammonium phosphate crystals bear a superficial resemblance to particles of broken glass, a close examination with a magnifying glass usually shows the difference. Struvite crystals occur most often in the form of regularly shaped prisms, with the edges tending to form straight lines. Glass particles are more likely to be irregular in shape.

3.4.5 Control of physical hazards

To determine whether particles are struvite or glass, the particles can be placed in warm household vinegar for 5–10 min. If the particles are struvite, they will dissolve fairly quickly.

Possible control measures may include: visual inspection, the use of filters and sieves, metal detectors, magnets, x-rays, separation by density and personal precautions of the personnel.

Control measures for “metal inclusion” can include: periodic checking of cutting or blending equipment or wire-mesh belts for damage or missing parts, and the passing of the product through metal detection or separation equipment.

Control measures for “glass inclusion” can include: visual examination of empty glass containers; cleaning (by water or compressed air) and inverting of empty glass containers; periodic monitoring of processing lines for evidence of glass breakage; proper adjustment of capping equipment; visual examination of glass containers containing transparent liquid fishery products; and the passing of the product through x-ray equipment or other defect rejection.

X-rays can also be used to detect other non-metallic objects.

4. Characterization of seafood spoilage and other quality issues

While the concepts of farm-to-fork in risk assessments have been developed to ensure food safety, the same approach and thinking process can also be applied to cover other quality aspects (where public health is not in question), such as sensory or nutritional quality, composition or labelling. Instead of identifying the hazards of the process/product, potential defects are considered.

A defect is defined as a condition found in a product that fails to meet essential quality, composition and/or labelling provisions of the appropriate product standard. These may be national regulations or commercial specifications or international Codex standards.

End-product specifications outlined in Appendixes II–XI of the Codex Code of Practice for Fish and Fishery Products describe optional requirements that are intended to assist buyers and sellers in describing those provisions that are often used in commercial transactions or in designing specifications for final products. These requirements are intended for voluntary application by commercial partners and not for systematic application by governments.

Similarly to the hazard analysis carried out to ensure food safety using HACCP, biological, chemical or physical agents capable of causing quality loss in a particular seafood or a seafood category need to be identified, and the qualitative and/or quantitative evaluation of the nature of the quality loss associated with those agents needs to be characterized. This assists in assessing the significance of the defect, namely its probability of occurrence and the adverse effect the defect will have on the product. The concept of a critical control point (CCP) is replaced by the concept of a defect action point (DAP).

Spoilage is responsible for important and costly post-harvest losses in fisheries and aquaculture. It has been estimated that the economic cost of losses in selected fisheries in selected countries in Africa ranges from US\$20 000 to US\$60 million – with quality issues accounting for up to 70 percent of the total losses (Akande and Diei-Ouadi, 2010). Consequently, implementing food quality management systems along the value chain to reduce post-harvest losses will not only increase fish and seafood supply for human consumption but also reduce the pressure on the wild-capture fish stocks and improve their sustainability. Likewise, the substitution of one (low-value) fish species for another (high-value) species is an example of a biological defect and an economic fraud. Abnormal water addition that brings about excessive fluid loss and shrinkage of frozen seafood products when thawed and/or cooked is another example of a physical defect resulting in economic fraud and loss of nutrients.

4.1 FISH SPOILAGE

Fresh fish is highly perishable and can have a short storage life. Several factors may affect the quality of fish as a raw material for the processing industry or as food for human consumption. Spoilage of fresh fish is predominantly the result of microbial activity, although not always, depending on the species. Physical damage due to rough handling may affect the fish integrity and may result in quality loss or total fish loss; it also predisposes fresh fish to accelerated water loss as well as autolytic activities, opportunistic infection and oxidation reactions during subsequent operations. Reactions in fish lipids can lead to quality deterioration, especially in frozen and dried

fish. Regardless of the cause, spoilage results in sensory changes that largely determine the perception of product quality by consumers.

4.1.1 Autolytic changes (Henri Loreal)

It has been known for many years that there are at least two types of fish spoilage: bacterial and enzymatic. Enzymatic activities are responsible for autolytic changes that occur in the first days after the death of the fish.

Autolysis means “self-digestion”. Uchiyama and Ehira (1974) showed that, for cod and yellowfin tuna, enzymatic changes affecting fish freshness preceded and were unrelated to changes caused by bacteria. In other species (squid and herring), the enzymatic changes precede and predominate in the spoilage pattern of chilled fish. In yet other fish species, autolysis contributes in varying degrees to the overall quality loss, in addition to microbially mediated processes.

4.1.1.1 Glycolysis – degradation of adenosine triphosphate

Post-mortem glycolysis results in the accumulation of lactic acid, which in turn lowers the pH of the muscle. This leads to a reduction in the net surface charge on the muscle proteins, causing them to denature partially and lose some of their waterholding capacity.

After death, rigor mortis sets in and the muscle adenosine triphosphate (ATP) level drops to about 1.0 $\mu\text{moles/g}$. This ultimately results in the shortening of the muscle, making it stiff and inextensible. A fish in rigor mortis cannot normally be filleted or processed because the carcass is too stiff to be manipulated and it is often contorted, making mechanical handling impossible.

The resolution of rigor mortis results in the subsequent softening (relaxation) of the muscle tissue, which is coincidental with the autolytic changes. One of the first autolytic changes to be studied after fish death was the degradation of ATP-related compounds.

$$K \text{ value (\%)} = \frac{[\text{Ino}] + [\text{Hx}] \times 100}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{Ino}] + [\text{Hx}]}$$

Where [ATP], [ADP], [AMP], [IMP], [Ino] and [Hx] represent the relative concentrations of ATP, adenosine diphosphate, adenosine monophosphate, inosine monophosphate, inosine and hypoxanthine in fish muscle measured at various times during chilled storage.

The K value, or “freshness” index, gives a relative freshness rating based primarily on the autolytic changes that take place in the muscle during fish storage. However, some fish species such as Atlantic cod reach a maximum K value well in advance of the end of shelf-life as determined by trained judges using sensory assessment techniques. Therefore, the K value is not considered reliable as a freshness index for all marine finfish.

Experiments on Japanese common squid (*Todarodes pacificus*) suggest that the increase in the content of ribose derived from post-mortem degradation of ATP and its related compounds is responsible for the browning of boiled, dried and seasoned squid products (sakiika or ikakun in Japanese). Thus, it is important to maintain freshness of squid as raw material for products to avoid browning during subsequent preservation of the product (Omura *et al.*, 2007).

4.1.1.2 Autolytic changes involving proteolytic enzymes

Many proteases have been isolated from fish muscle, and the effects of proteolytic breakdown are often related to extensive softening of the tissue. Perhaps one of the most notable examples of autolytic proteolysis is the incidence of belly-bursting in

pelagic (fatty-fish) species such as herring and capelin. This type of tissue softening is most predominant in summer months when pelagics are feeding heavily, particularly on “red feed” consisting of copepods and euphausiids.

4.1.1.3 *Cathepsins*

The cathepsins are “acid” proteases usually found packaged in tiny, submicroscopic organelles called lysosomes. Cathepsin L causes muscle softening, mainly in frozen/thawed tissue.

4.1.1.4 *Calpains*

The calpains are intracellular endopeptidases. Calpains have been found primarily responsible for the post-mortem autolysis of meat through digestion of the z-line proteins of the myofibril. Most calpains are active at physiological pH, making it reasonable to suspect their importance in muscle softening during chilled storage. Fish species adapted to colder environmental temperatures are more susceptible to calpain autolysis than those from tropical waters.

4.1.1.5 *Collagenases*

Instrumental measurements of texture of chilled trout muscle showed a texture deterioration as the amount of type V collagen was solubilized, presumably due to the action of autolytic collagenase enzymes (Sato *et al.*, 1991). These enzymes are likely to be the cause of “gaping” or breakdown of the myotome during long-term fish storage on ice or short-term storage at high temperature.

The relatively short shelf-life of chilled prawns, due to softening of the tissue, has also been shown to result from the presence of collagenase enzymes (Nip, Lan and May, 1985). The source of the collagenase enzymes in prawn is thought to be the hepatopancreas (digestive organ).

4.1.1.6 *Autolytic changes during frozen storage*

The reduction of trimethylamine oxide (TMAO), an osmoregulatory compound in many marine teleost fish, is usually due to bacterial action. However, in some species, an enzyme is present in the muscle tissue that is able to break TMAO down into dimethylamine (DMA) and formaldehyde. Formaldehyde induces cross-linking of the muscle proteins, which makes the muscle tough and reduces its waterholding capacity. The enzyme responsible for formaldehyde-induced toughening is called TMAO-ase or TMAO demethylase and is most commonly found in the gadoid fishes (cod family).

Most of the TMAO demethylase enzymes reported to date have been membrane-bound. They become most active when the tissue membranes are disrupted by freezing or artificially by detergent solubilization. Dark (red) muscle has a higher rate of activity than white muscle, whereas other tissues such as fish kidney, spleen and gall bladder are extremely rich in the enzyme. Thus, it is important that minced fish is completely free of organ tissue, such as kidney from gadoid species, if toughening in frozen storage is to be avoided.

The most practical means of preventing the autolytic production of formaldehyde in frozen fish is to store fish at temperatures of less than -30°C , to minimize temperature fluctuations in the cold store and to avoid rough handling or the application of physical pressure on the fish prior to freezing.

The autolytic changes affecting the edibility of fresh and frozen fish are summarized in Table 56. Generally, the most important single factor leading to autolysis is physical disruption of the muscle cells. Many of the autolytic enzymes have been shown to be compartmentalized in discrete membrane-bound packages, which become broken when subjected to physical abuse and result in the intimate mixing of enzyme and substrate. Crushing of the fish by ice or other means can seriously affect the edibility

and filleting yields even for fish that have a relatively low bacterial load, demonstrating the importance of autolytic processes.

TABLE 56

Summary of autolytic changes in chilled fish

Enzyme	Substrate	Changes encountered	Prevention/inhibition
Glycolytic enzymes	Glycogen	Production of lactic acid, pH of tissue drops, loss of waterholding capacity in muscle High-temperature rigor may result in gaping	Fish should be allowed to pass through rigor at temperatures as close to 0 °C as practically possible Pre-rigor stress must be avoided
Autolytic enzymes, involved in nucleotide breakdown	ATP, ADP, AMP, IMP	Loss of fresh fish flavour, gradual production of bitterness with Hx (later stages)	Same as above Rough handling or crushing accelerates breakdown
Cathepsins	Proteins, peptides	Softening of tissue, making processing difficult or impossible	Rough handling during storage and discharge
Chymotrypsin, trypsin, carboxy-peptidases	Proteins, peptides	Autolysis of visceral cavity in pelagics (belly-bursting)	Problem increased with freezing/thawing or long-term chill storage
Calpain	Myofibrillar proteins	Softening, moult-induced softening in crustaceans	Removal of calcium thus preventing activation?
Collagenases	Connective tissue	"Gaping" of fillets, softening	Connective tissue degradation related to time and temperature of chilled storage
TMAO demethylase	TMAO	Formaldehyde-induced toughening of frozen gadoid fish	Store fish at temperature ≤ -30 °C Physical abuse and freezing/thawing accelerate formaldehyde-induced toughening

4.1.2 Microbiological changes (Paw Dalgaard)

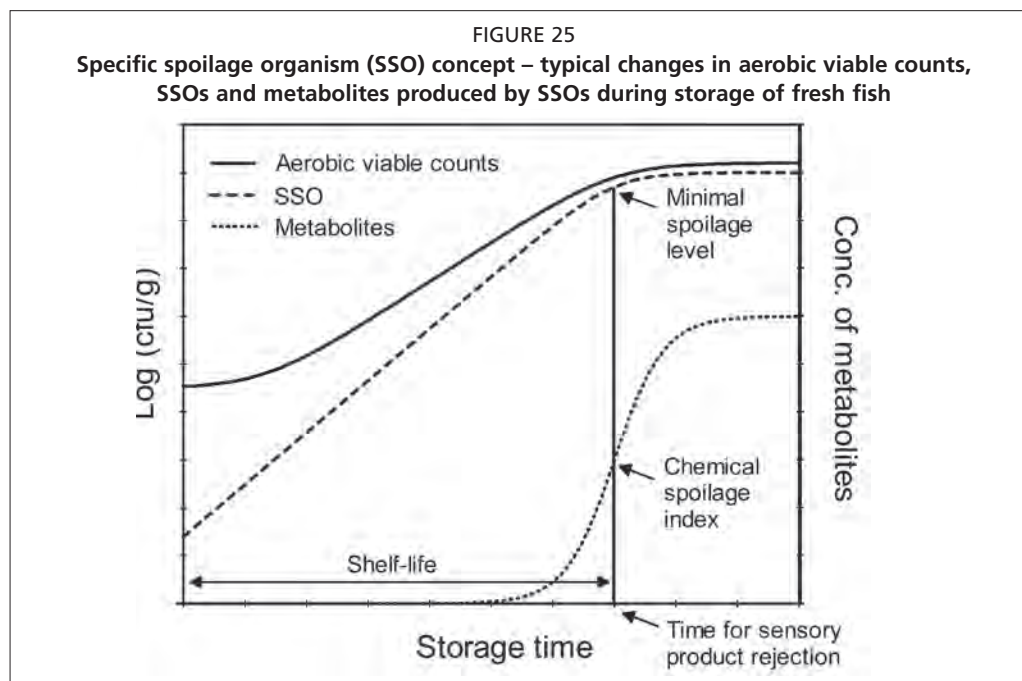
The presence, growth and activity of micro-organisms in raw material and products of finfish, crustaceans and molluscan shellfish influence spoilage and shelf-life. Assessment and management of relevant micro-organisms are important to avoid defects and problems with shelf-life. Only some of the numerous species of micro-organisms in aquatic muscle food are important for spoilage, and, during storage, a pattern of microbial growth and activity, called the specific spoilage organism (SSO) concept, is frequently observed. In newly processed, fresh or lightly preserved fish muscle, the SSOs are usually present in very low concentrations and constitute only a minor part of the total microflora. Then, during storage, the SSOs grow faster than the remaining microflora, produce metabolites responsible for off-flavours, and finally cause sensory product rejection (Figure 25). The SSOs often consist of a single or a few microbial species, whereas the microflora found in a product at the time sensory assessments indicate spoilage typically include several groups of micro-organisms. The SSOs are typically present in concentrations of about 10⁷ cells/g or colony forming units per gram when sensory spoilage becomes apparent.

This section briefly describes the occurrence, growth and activity of micro-organisms in fish and seafood muscle, and illustrates how information about SSOs can be used to determine, predict and extend the shelf-life of products.

4.1.2.1 Microflora of aquatic animals from different habitats

The microflora of aquatic animals at capture or harvest depends to a large extent on the microflora of the water in which they live. Micro-organisms are found on outer surfaces (e.g. skin, gills and intestine), whereas the muscle tissue of healthy aquatic animals is sterile. The concentration of culturable micro-organisms is variable and, in general, 10²–10⁵ cfu/cm² are found on skin, 10³–10⁷ cfu/g in gills and 10²–10⁸ cfu/g in the intestines. The variable concentrations of intestinal micro-organisms are related to the aquatic animal's intake of food. Higher temperatures typically correspond to higher concentrations of culturable micro-organisms in water and on aquatic animals, whereas

water salinity has little effect on the total concentration of micro-organisms (Horsley, 1977; Liston, 1980; Cahill, 1990; Yoshimizu and Kimura, 1976; Okuzumi and Awano, 1983; Yoguchi, Okuzumi and Fujii, 1990). Regarding the effect of catching methods, trawled finfish may have 10–100 times higher concentrations of micro-organisms on skin and gills than similar fish caught by longline (Shewan, 1961).



Source: Modified from Dalgaard (2000).

The genera or groups of micro-organisms found on skin, on the outer shell and in gills of newly caught or harvested finfish, crustaceans and shellfish have been extensively studied. The dominating groups of Gram-negative bacteria are: (i) *Acinetobacter* and *Moraxella/Psychrobacter*; (ii) *Pseudomonas* and *Shewanella*; (iii) *Flavobacterium* and *Cytophaga*; (iv) *Vibrio* and *Photobacterium*; (v) *Aeromonas*; and (vi) Enterobacteriaceae. The dominating groups of Gram-positive bacteria are cocci, primarily *Micrococcus*, coryneforms and rods including *Bacillus*, *Clostridium* and lactic-acid bacteria. When data from many studies are compared, little can be concluded about the effect of water temperature and salinity or about the type of animal, for example demersal or pelagic, on the percentage distribution of genera and/or groups of micro-organisms. However, for animals in freshwater, the sodium-requiring species of *Vibrio* and *Photobacterium* are very rarely present, whereas *Aeromonas* and Enterobacteriaceae are relatively more important in those habitats. Moreover, the *Flavobacterium-Cytophaga* group seems less dominant in marine animals. Furthermore, the percentages of *Bacillus*, *Micrococcus* and Enterobacteriaceae tend to be higher in tropical than in temperate regions. The apparent lack of difference between groups of micro-organisms on aquatic animals from various habitats may result from the use of simple identification schemes relying on relatively few phenotypic characteristics (Horsley, 1977; Shewan, 1962; Liston, 1980; Cahill, 1990; Karunasagar and Karunasagar, 1991; Gram and Huss, 2000).

Owing to the high concentration of intestinal micro-organisms in aquatic animals, contamination of products during processing is important and difficult to avoid. Marine fish with a developed digestive tract have a specific gut microflora consisting of marine vibrios including *Photobacterium phosphoreum*. Fish with a simple digestive tract, e.g. immature individuals, have more complex intestinal flora that reflect the microflora in water and feed (Sera and Ishida, 1972; Yoshimizu and Kimura, 1976).

Data from several studies have shown that *Vibrio/Photobacterium*, *Pseudomonas* and Enterobacteriaceae dominate the intestinal microflora of marine fish species, whereas Enterobacteriaceae, *Aeromonas* and *Pseudomonas* dominate in the intestinal content of freshwater species. However, *Acinetobacter/Moraxella*, lactic-acid bacteria, yeasts and strictly anaerobic micro-organisms, including *Bacterioides* and *Clostridium*, can occur in high concentrations. In addition, a *Mycoplasma* phenotype has been determined in salmon by a culture-independent approach relying on extraction and amplification of 16S rDNA (Horsley, 1977; Cahill, 1990; Ringø, Strøm and Tabachek, 1995; Ringø and Gatesoupe, 1998; Spanggaard *et al.*, 2001; Holben *et al.*, 2002; Hovda *et al.*, 2007).

4.1.2.2 Microbial spoilage of aquatic muscle food

Newly caught fish and shellfish typically have a species-specific flavour that disappears after a few days of chilled storage. Further storage results in development of off-flavours, which are often ammonia-like, sulphurous, malt-like or rancid. The importance of microbial activity in seafood spoilage has been established by comparing off-flavour development in muscle pieces that were: (i) sterile, (ii) inoculated with specific micro-organisms, or (iii) naturally contaminated. These studies showed that the short shelf-life of many products is explained by their microflora and chemical characteristics.

Numerous fish and other aquatic animals of technological importance live in cold waters, and their natural microflora include psychrotolerant species able to grow readily in chilled products at temperatures above -2°C to 0°C . This explains the relatively short shelf-life of 12–18 days for many coldwater fish when stored in ice whereas the corresponding shelf-life for tropical white-fleshed fish is typically 18–35 days at 0°C (Gram, 1989; Dalgaard and Huss, 1997). In addition, the flesh of some fishes, crustaceans and molluscs contains trimethylamine oxide (TMAO), which stimulates microbial growth and activity. In general, animals from freshwater contain less TMAO than those from seawater, but considerable variation exists between species in both habitats (Hebard, Flick and Martin, 1982). *Aeromonas*, *Alteromonas*, most Enterobacteriaceae, *Shewanella* and *Vibrio* and *Photobacterium*, including all marine luminous bacteria, reduce TMAO to trimethylamine (TMA). This anaerobic respiration facilitates their growth under oxygen-limiting conditions, e.g. in vacuum-packed or modified-atmosphere packed products (Barret and Kwan, 1985; Proctor and Gunasalus, 2000). Trimethylamine contributes to the typical ammonia-like and fishy off-odours in spoiled seafoods, particularly in products with pH above ~6.5 (Castell and Triggs, 1955). Moreover, the post-rigor pH of finfish, crustaceans and molluscs is high compared with beef and pork. Again, this contributes to a short shelf-life. White-fleshed demersal finfish and crustaceans have a pH of ~6.5 to above 7, whereas pelagic, dark-fleshed fish such as tuna, mahi-mahi, mackerel and garfish, have a pH as low as ~5.8. Molluscs have pHs similar to white-fleshed finfish, but they contain much more carbohydrate (2.5–5.0 percent) as compared with the < 0.5 percent for finfish and crustaceans. Consequently, a fermentative type of spoilage with decreasing pH is typical for molluscs but most unusual in other seafoods unless carbohydrates are added (Bremner and Statham, 1983; ICMSF, 1998; López-Caballero *et al.*, 2000; He *et al.*, 2002; Vasakou, Vareltzis and Bloukas, 2003). Finally, high concentrations of free amino acids are present in seafoods and metabolized by spoilage micro-organisms, e.g. arginine in shrimps, and histidine in dark-fleshed pelagic finfish (Abe, 1983; Chinivasagam *et al.*, 1998).

It is well established that many micro-organisms from seafoods produce extracellular proteolytic enzymes (Venugopal, 1990; Kobatake *et al.*, 1992). Nevertheless, seafood-spoilage micro-organisms typically produce off-flavours from substrates in muscle extractives, and proteolytic activity is not important for spoilage of fresh seafoods (Lerke, Farber and Adams, 1967; Karnop, 1982). The importance of microbial

proteolytic enzymes has primarily been evaluated for fresh fish, and further research, including on lightly preserved and semi-preserved seafoods, seems justified.

4.1.2.3 Specific spoilage organisms in groups of aquatic muscle food

Spoilage of fresh chilled and aerobically stored seafood is primarily caused by H₂S-producing *Shewanella* bacteria and *Pseudomonas* spp. This is well established (Table 57), but the taxonomy of these Gram-negative and non-fermentative rods has been changing. In fish products, H₂S-producing *Shewanella* bacteria have often been isolated as black colonies using pour plating in iron agar, and then identified as *Shewanella putrefaciens* by a limited number of phenotypic tests. It has been shown more recently that these H₂S-producing bacteria consist of a number of *Shewanella* species and that *S. baltica* is common in several types of fish products (Ziemke, 1998; Stenström and Molin, 1990; Fonnesbech Vogel *et al.*, 2005; Satomi *et al.*, 2006).

Frequently, *Pseudomonas* have not been identified at the species level, but strains similar to *Ps. fragi*, *Ps. fluorescens* and *Ps. putida* seem common in seafood, and *P. lundensis* dominates the spoilage microflora of chilled aerobically stored marine fish from Greece (Gillespie, 1981; Tryfinopoulou, Tsakalidou and Nychas, 2002; Stenström and Molin, 1990). *Pseudomonas* spp. are unable to reduce TMAO, and growth is considerably reduced under oxygen-limited conditions. Other bacteria may also influence spoilage of fresh chilled and aerobically stored seafood. Thus, *P. phosphoreum* can be responsible for TMA production and contribute to spoilage of various chilled fish stored aerobically. See Dalgaard (1998) for a review and also more recent studies of different fish species (Dalgaard *et al.*, 2006; Olafsdottir *et al.*, 2006a; Olafsdottir *et al.*, 2006b). In addition, lipolytic *Psychrobacter immobilis* can dominate the spoilage microflora in both marine and freshwater fish and, despite a low spoilage potential, they may increase the rancid spoilage of sardines (Gennari, Tomaselli and Cotrona, 1999; González *et al.*, 2000).

For fresh seafood in MAP with high concentrations of CO₂, luminous and non-luminous variants of *P. phosphoreum* are important spoilage micro-organisms (Table 57). The *P. phosphoreum* species group is heterogeneous and isolates from seafood are likely to belong to several species, including *P. iliopiscarium* (Dalgaard, Manfio and Goodfellow, 1997; Ast and Dunlap, 2005; Olofsson, Ahrné and Molin, 2007). The relative importance of *P. phosphoreum* and H₂S-producing *Shewanella* species in vacuum-packed fresh chilled seafoods probably depends on the initial concentration of the two spoilage bacteria. For shucked bivalve molluscs, i.e. mollusc meat removed from the shells, spoilage is fermentative when the product is stored under vacuum or otherwise with reduced access to oxygen, but the micro-organisms responsible, remain to be identified (Bremner and Statham, 1983; Kim, Paik and Lee, 2002; Vasakou, Vareltzis and Bloukas, 2003). Spoilage of chilled MAP seafood from freshwater or products without TMAO need further study, for example, with respect to identification of lactic-acid bacteria and the importance of *Aeromonas* spp. (Table 57). High concentrations of *Aeromonas* spp. have been found in chilled MAP seafood from tropical regions and they are likely to be the SSO (Table 57).

For chilled lightly preserved seafood, lactic-acid bacteria seem to be the most important groups of spoilage micro-organisms (Table 57). However, identification of the SSO responsible for spoilage has been complicated, and variation in product characteristics, including the initial microflora, NaCl, pH, smoke components, chemical preservatives and packaging, is probably responsible for the various spoilage patterns observed (Table 57). *Staphylococcus xylosus*, *Halobacterium salinarium* and moulds have been suggested as spoilage organisms for sun-dried tropical fish depending on storage temperature and water activity (Doe and Heruwati, 1988).

Micro-organisms in seafoods can interact in several ways, including substrate competition and metabolite inhibition (Jørgensen, Huss and Dalgaard, 2000;

Gram *et al.*, 2002). This may influence their spoilage activity and Joffraud *et al.* (2001) found mixtures of different spoilage bacteria form stronger off-odours and higher concentrations of specific metabolites than mixtures of strains from individual species. The importance of such interactions on spoilage and shelf-life of seafood warrants further study.

TABLE 57

Specific spoilage organisms in groups of fresh and lightly preserved seafood

Seafood	Typical specific spoilage organisms	Metabolites produced
Fresh chilled products stored in air		
Various species, particularly those containing TMAO ^c and with pH above 6	H ₂ S-producing <i>Shewanella</i> ^a	TMA [*] , hydrogen sulphide and other sulphur compounds, hypoxanthine
Various species, including some with little or no TMAO and low pH of about 6	<i>Pseudomonas</i> spp.	Ammonia, esters, sulphur compounds but not hydrogen sulphide
Fresh, chilled products in modified atmosphere packaging		
TMAO containing species from seawater at temperatures below ~15 °C	<i>Photobacterium phosphoreum</i>	TMA, hypoxanthine, alcohols, ketones and biogenic amines
Species from warmer waters, particularly species with little or no TMAO	Lactic-acid bacteria and <i>Brochothrix thermosphacta</i> ^b	Acetic acid, ammonia, tyramine, acetoin, diacetyl, hydrogen sulphide
Species from tropical freshwater	<i>Aeromonas</i> spp.	?
Fresh and lightly preserved products stored at ambient temperature	<i>Aeromonas</i> spp.	TMA, sulphur compounds, biogenic amines
	<i>Vibrio</i> spp. / <i>Photobacterium</i> spp. Enterobacteriaceae <i>Enterococcus faecalis</i>	NH ₃ , acetic acid, acetoin, diacetyl, tyramine
Lightly preserved and chilled products	Lactic-acid bacteria ^c and <i>Brochothrix thermosphacta</i>	Acetic acid, ammonia, tyramine, acetoin, diacetyl, sulphur compounds
Brined, spiced/gravad and smoked products, including fish roe	<i>P. phosphoreum</i> , <i>Vibrio</i> and Enterobacteriaceae ^d	TMA, biogenic amines, alcohols, aldehydes, sulphur compounds

^a *Shewanella putrefaciens*, *Shewanella baltica* and other closely related H₂S-producing Gram-negative bacteria.

^b *Brochothrix thermosphacta* is important for products in oxygen-containing modified atmospheres.

^c Include *Lactobacillus curvatus*, *Lactobacillus sake* and *Leuconostoc* spp.

^d Include *Enterobacter agglomerans*, *Hafnia alvei* and *Serratia liquefaciens*.

* TMA = trimethylamine; TMAO = trimethylamine oxide.

Source: Modified from Dalgaard (2006).

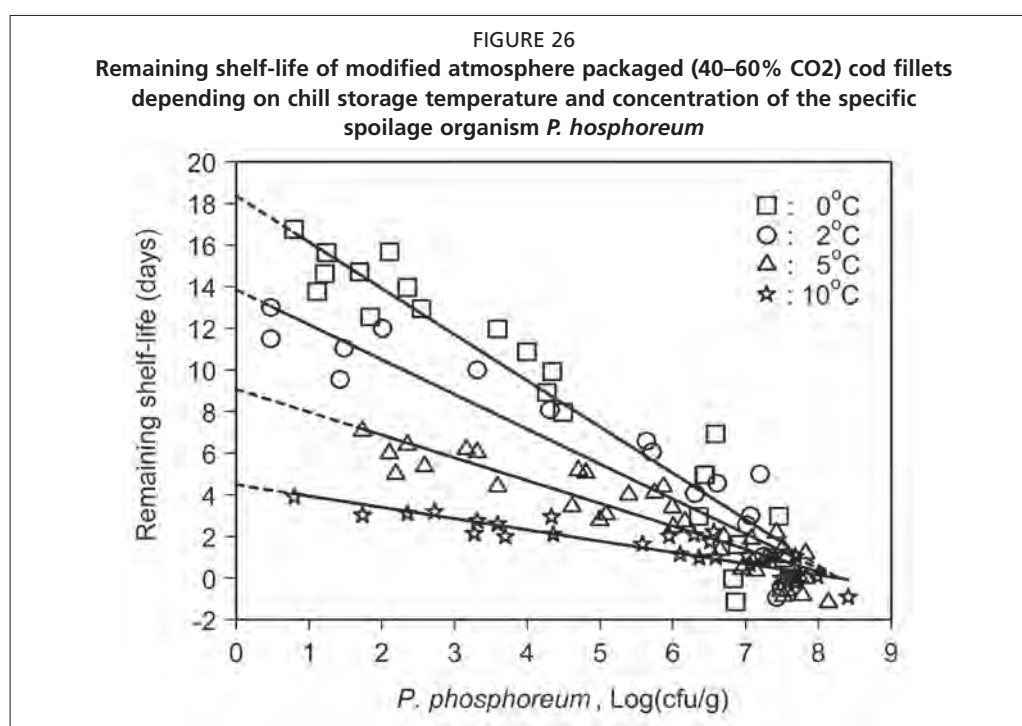
4.1.3 Determination, prediction and extension of shelf-life (Paw Dalgaard)

Indices of freshness or spoilage of seafood have been extensively studied, and it is generally accepted that there is a poor correlation between remaining shelf-life, as determined by sensory methods, and aerobic viable counts (Ólafsdóttir *et al.*, 1998). Nevertheless, the aerobic viable count is used to evaluate the hygienic status of aquatic muscle foods. Heat-labile and sodium-requiring micro-organisms are common in products from seawater and brackish waters (Dalgaard, 2006). For enumeration of these micro-organisms, pour plating with ~45 °C warm agar must be avoided as this procedure may kill a major part of the microflora. The concentration of micro-organisms in deep-water pink shrimp (*Parapenaeus longirostris*) is, for example, about 20 times higher when determined by spread plating as compared with pour plating. Moreover, micro-organisms on seafood frequently require sodium for growth and, although standard plate count agar without NaCl is recommended for many foods, this medium is inappropriate for seafood. For various fresh and lightly preserved seafoods, spread plating on pre-chilled plates of Long and Hammer's agar with 1 percent NaCl, aerobically incubated for 5–7 days at 15 °C, has been appropriate for enumeration of the dominating microflora (van Sreeken, 1974; Dalgaard, 2000;

NMKL, 2006; López-Caballero, Goncalves and Nunes, 2002). Microbiological criteria relying on mesophilic aerobic bacteria in concentrations between 10^5 and 10^6 cfu/g are included in regulatory frameworks. However, owing to the frequent dominance of heat-labile spoilage bacteria in seafood, it must not be expected that such criteria correspond to sensory spoilage.

In contrast to the situation for aerobic viable counts, close correlations (correlation coefficients between -0.929 and -0.975) have been observed between remaining shelf-life and concentrations of SSOs in different chilled fish products (Capell, Vaz-Pires and Kirby, 1998; Dalgaard, 1998; Koutsoumanis *et al.*, 1998). Thus, as shown in Figure 26 for *P. phosphoreum* in cod fillets, the concentration of an SSO can be used to estimate the remaining shelf-life at different chill storage temperatures.

For lightly preserved seafood, including cold-smoked and marinated products, simple correlations have not typically been observed between the remaining shelf-life and concentrations of any specific group of micro-organisms or any specific metabolite. However, the remaining shelf-life or sensory quality of some of these products can be related with so-called multiple-compound quality indices to concentrations of several microbial metabolites and/or specific micro-organisms (Jørgensen, Dalgaard and Huss, 2000; Leroi *et al.*, 2001).



Source: Dalgaard (1998).

Specific spoilage organisms often grow without a lag phase in fresh fish products, and this facilitates shelf-life prediction (Figure 26). In fact, kinetic models have been developed to predict growth of *B. thermosphacta*, *P. phosphoreum*, psychrotolerant *Pseudomonas* spp. and H₂S-producing *Shewanella* as a function of storage conditions (atmosphere and/or temperature). In addition, the models have been incorporated in user-friendly application software. This makes it convenient to evaluate the effect on shelf-life of product temperature profiles recorded by data-loggers. See Dalgaard (2002)⁸

⁸ The Seafood Spoilage and Safety Predictor (SSSP) software is available free of charge at: <http://sssp.dtuqua.dk>

and specific studies (Koutsoumanis and Nychas, 2000; Nuin *et al.*, 2008). Increased transportation of seafood nationally and internationally makes this type of time-temperature integration important to preventing disappointed consumers and rejections of products. Other micro-organisms, including species of *Aeromonas*, Enterobacteriaceae, lactic-acid bacteria and *Vibrio*, are also important in seafood spoilage (Table 57). For these SSOs, shelf-life models remain to be developed and/or validated in relevant seafoods. Another and more challenging future task is to predict the species of micro-organisms that become SSOs when: (i) new products are formulated; (ii) seafoods are processed by a new technology; or (iii) seafoods are stored under conditions not previously evaluated.

Extension of shelf-life is important for various aquatic-muscle foods, and both classical technologies (e.g. reduced temperature, MAP, salting, smoking and addition of antimicrobials) and newer methods (e.g. biopreservation, high-pressure processing and pulsed light) are interesting for different types of products. Inhibiting or preventing growth of an SSO results in increased shelf-life, and the extension is proportional to the growth delay. However, when growth of an SSO is markedly reduced, another SSO or another spoilage reaction (biochemical, chemical or physical) will determine shelf-life. The targeted inhibition of SSOs is interesting as product shelf-life may be extended by mild preservation methods, selected depending on properties of a particular SSO. However, the effect on shelf-life of inhibiting growth of a particular SSO must be determined using storage trials and sensory evaluation. As one example, the chilled shelf-life of frozen and thawed MAP cod, salmon and garfish is extended, compared with the fresh products, when the SSO (*P. phosphoreum*) is inactivated by frozen storage (Guldager *et al.*, 1998; Emborg *et al.*, 2002; Bøknæs *et al.*, 2002; Dalgaard *et al.*, 2006). For Nile perch at ambient temperature, spoilage is due to mesophilic motile *Aeromonas* spp. and inhibiting this SSO, using combinations of NaCl, sorbate and smoke components, resulted in a lightly preserved fish product with a marked shelf-life extension (Gram, 1991).

4.1.4 Lipid oxidation and hydrolysis (Henri Loreal)

Two important reactions can take place in fish lipids, and both lead to quality deterioration. They are oxidation and hydrolysis, and they result in the production of a range of substances, some of which have an unpleasant (rancid) taste and smell, while others contribute to texture changes by binding covalently to fish-muscle proteins. The various reactions are either non-enzymatic or catalyzed by microbial, intracellular or digestive enzymes from the fish tissues. The relative significance of these reactions depends mainly on the fish species and storage temperature.

Fatty fish are particularly susceptible to lipid degradation, which can create severe quality problems even for storage at temperatures below zero.

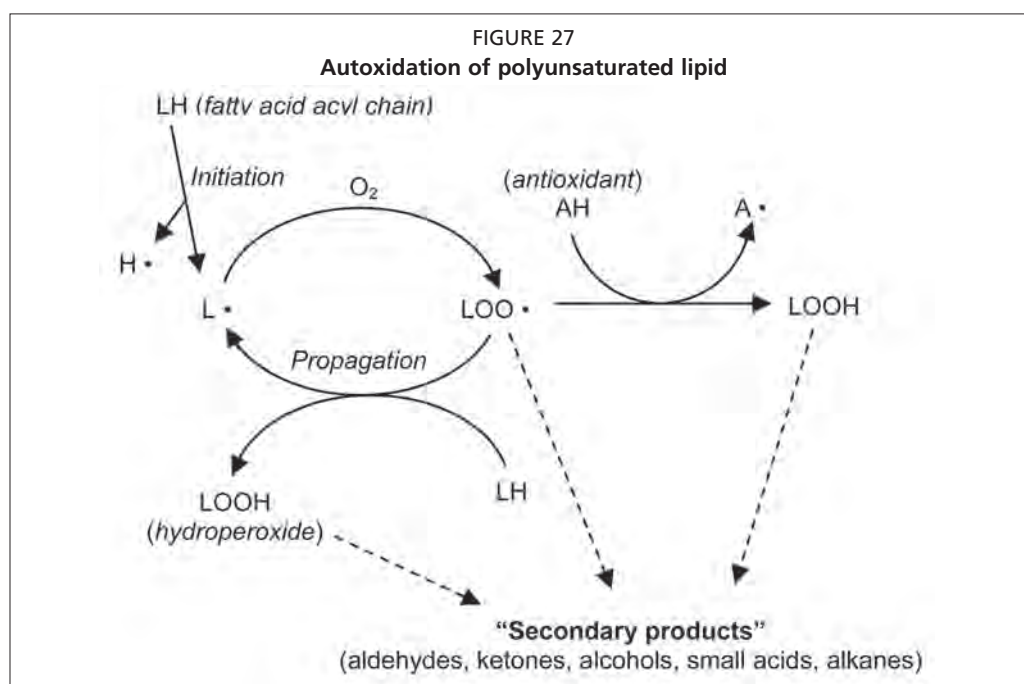
4.1.4.1 Lipid oxidation

The large amount of polyunsaturated fatty acid moieties found in fish lipids makes them highly susceptible to oxidation by an autocatalytic mechanism (Figure 27). The process is initiated as described below by abstraction of a hydrogen atom from the central carbon of the pentadiene structure found in most fatty acid acyl chains containing more than one double bond:



Contrary to the native molecule, the lipid radical (L) reacts very quickly with atmospheric oxygen making a peroxy-radical (LOO), which again may abstract a hydrogen from another acyl chain resulting in a lipid hydroperoxide (LOOH) and a new radical L. This propagation continues until one of the radicals is removed by

reaction with another radical or with an antioxidant (AH) resulting in a radical (A) that is much less reactive.



The hydroperoxides are readily broken down to secondary autoxidation products of shorter carbon chain-length. This reaction is catalyzed by heavy metal ions. The resulting secondary products – mostly aldehydes, ketones, alcohols, small carboxylic acids and alkanes – give rise to a very broad odour spectrum and in some cases to a yellowish discoloration. Several of the aldehydes can be determined as “thiobarbituric acid-reactive substances”.

Lipid oxidation of the dark muscle has been shown to be closely related to meat darkening and development of the rancid off-odour during the early stage of ice storage of cultured yellowtail, *Seriola quinqueradiata* (Sohn *et al.*, 2005).

4.1.4.2 Hydrolysis

During storage, a considerable amount of free fatty acids develop. The phenomenon is more profound in ungutted than in gutted fish, probably because of the involvement of digestive enzymes. Triglyceride in the depot fat is cleaved by triglyceride lipase originating from the digestive tract or excreted by certain micro-organisms. Cellular lipases may also play a minor role.

In lean fish, for example, Atlantic cod, production of free fatty acids also occurs even at low temperatures. The fatty acids give a “soapy” off-flavour to the fish.

4.1.5 Sensory changes (Henri Loreal)

All these microscopic changes, caused by bacteria, autolytic enzymes or other chemical reactions, are translated into changes in the sensory attributes of fish or seafood. Sensory changes are those perceived with the senses, i.e. appearance, odour, texture and taste.

4.1.5.1 Changes in raw fresh fish

The first sensory changes in fish during storage in ice are concerned with appearance and texture. The most dramatic change is onset of rigor mortis. Immediately after

death, the muscle is totally relaxed and the limp elastic texture usually persists for some hours, whereafter the muscle contracts. When it becomes hard and stiff, the whole body becomes inflexible and the fish is in rigor mortis. This condition usually lasts for a day or more in ice, and then rigor resolves. The resolution of rigor mortis makes the muscle relax again and it becomes limp, but no longer as elastic as before rigor. The rate in terms of onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical condition of the fish.

Abe and Okuma (1991) have shown that the onset of rigor mortis in carp (*Cyprinus carpio*) depends on the difference in sea temperature and storage temperature. When the difference is large, the time from death to onset of rigor is short and vice versa.

Rigor mortis starts immediately or shortly after death if the fish is starved and the glycogen reserves are depleted, or if the fish is stressed. The method used for stunning and killing the fish also influences the onset of rigor. Stunning and killing by hypothermia (the fish is killed in iced water) give the fastest onset of rigor, while a blow on the head gives a delay of up to 18 h (Azam, Mackie and Smith, 1990; Proctor, Ryan and McLoughlin, 1992).

The technological significance of rigor mortis is of major importance when the fish is filleted before or in rigor. In rigor, the fish body will be completely stiff, the filleting yield will be very poor, and rough handling can cause gaping. If the fillets are removed from the bone pre-rigor the muscle can contract freely and the fillets will shorten following the onset of rigor. Dark muscle may shrink by up to 52 percent and white muscle by up to 15 percent of the original length (Buttkus, 1963). If the fish is cooked pre-rigor, the texture will be very soft and pasty. In contrast, the texture is tough but not dry when the fish is cooked in rigor. Post-rigor, the flesh will become firm, succulent and elastic. Whole fish and fillets frozen pre-rigor can give good products if they are carefully thawed at a low temperature in order to give rigor mortis time to pass while the muscle is still frozen.

Freshness is the key element in the determination of the quality of fish by consumers. The sensory evaluation of raw fish in markets and landing sites is done by assessing the appearance, texture and odour. Most scoring systems are based upon changes taking place during storage in melting ice. It should be remembered that the characteristic changes vary depending on the storage method. The appearance of fish stored under chilled condition without ice does not change as much as for iced fish, but the fish spoil more rapidly and an evaluation of cooked flavour will be necessary. Knowledge of the time and temperature history of the fish should therefore be essential at landing.

The characteristic sensory changes in fish post-mortem vary considerably depending on fish species and storage method. The ratings established in regulations of the European Union (Member Organization) apply to the following products or groups of products, by reference to appraisal criteria specific to each of them:

- A. Whitefish: haddock, cod, saithe, pollock, redfish, whiting, ling, hake, Ray's bream, anglerfish, pouting and poor cod, bogue, picarel, conger, gurnard, mullet, plaice, megrim, sole, dab, lemon sole, flounder, scabbard fish.
- B. Bluefish: albacore or longfinned tuna, bluefin tuna, bigeye tuna, blue whiting, herring, sardines, mackerel, horse mackerel, anchovy, sprat.
- C. Selachii: dogfish, skate.
- D. Cephalopods: cuttlefish.
- E. Crustaceans: shrimps, Norway lobster.

The scale is numbered from 0 to 3, where 3 is the best quality.

However, this kind of scheme is limited when classifying the quality of some species. It does not take into account differences between species, and it only uses general parameters to describe the changes for iced fish.

These generally recognized limitations of the European Commission scheme and other previous schemes have necessitated the development of improved freshness

quality grading systems. One example is the quality index method (QIM), which is based upon objective evaluation of certain attributes of raw fish (skin, eyes, gills, etc.) using a demerit points scoring system (from 0 to 3). The scores are summarized to give the quality index, which increases linearly with the storage time in ice.

Publications on new or modified QIM schemes in peer-viewed journals or books cover at least 34 seafood species or products. For example, a QIM has been developed for gilthead seabream, *Sparus aurata* (Huidobro, Pastor and Tejada, 2000); more recently, Vaz-Pires and Seixas (2006) have developed sensory schemes for freshness grading of cuttlefish (*Sepia officinalis*) and broadtail shortfin squid (*Illex coindetii*). The shelf-life, as measured by sensory attributes, is considered to be 10 days in ice for cuttlefish and 9 days in ice for squid. For both species, a high correlation between the quality index and the storage time in ice was obtained.

A QIM has also been used to study the effects of short-time temperature abuse in the shelf-life of freshwater arctic char (*Salvelinus alpinus*) in relation to estimating the remaining storage time until sensory rejection (Cyprian *et al.*, 2008).

Most QIM schemes have been developed for whole raw fish, but several schemes have been developed for other types of seafood and products, such as raw, frozen/thawed fillets and cooked fillets. Multilingual guidelines and reference manuals for end users have been translated and published in 11 languages.⁹

4.1.5.2 Changes in eating quality

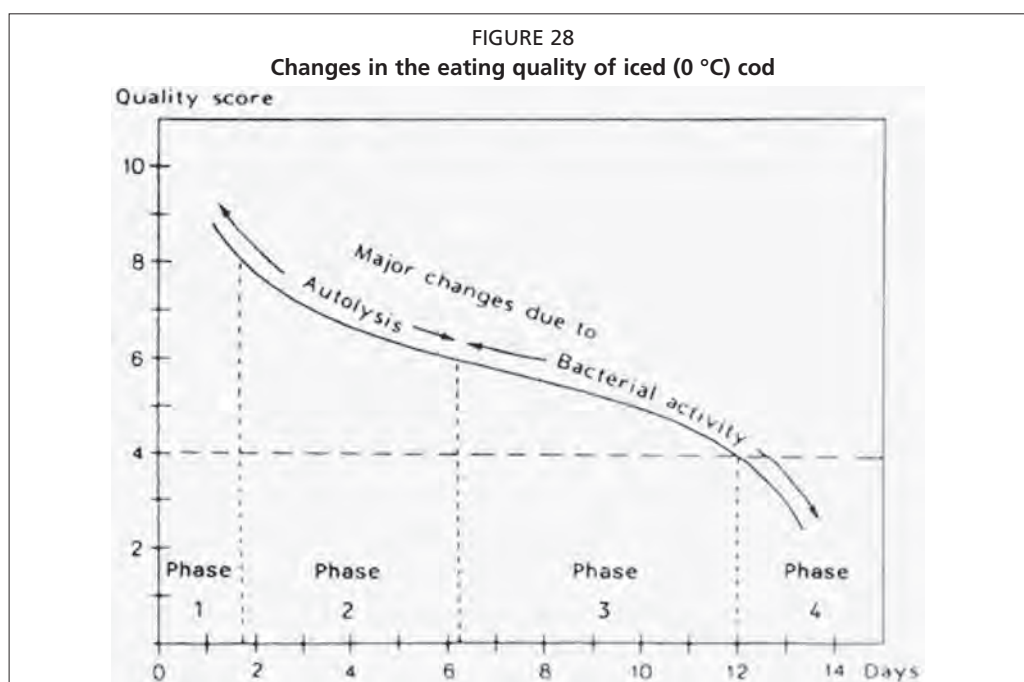
If knowledge on the eating quality of chilled fish during storage is required, a sensory assessment of the fish, cooked under controlled conditions, can be conducted. A characteristic pattern of the changes found in cooked fish has been elucidated and can be divided into the following four phases:

- **Phase 1.** The fish is very fresh and has a sweet, seaweedy and delicate taste. The taste can be very slightly metallic. In cod, haddock, whiting and flounder, the sweet taste is maximized 2–3 days after catching.
- **Phase 2.** There is a loss of the characteristic odour and taste. The flesh becomes neutral but has no off-flavours. The texture is still pleasant.
- **Phase 3.** There are signs of spoilage and a range of volatile, unpleasant-smelling substances is produced depending on the fish species and type of spoilage (aerobic, anaerobic). One of the volatile compounds may be TMA (derived from the bacterial reduction of TMAO), which has a characteristic “fishy” smell. At the beginning of this phase, the off-flavour may be slightly sour, fruity and slightly bitter, especially in fatty fish. In the later stages, sickly sweet, cabbage-like, ammoniacal, sulphurous and rancid smells develop. The texture becomes either soft and watery or tough and dry.
- **Phase 4.** The fish can be characterized as spoiled and putrid.

A numbered scale may be used for the sensory evaluation of cooked fish, as shown in Figure 28. The scale is numbered from 0 to 10, with 10 indicating absolute freshness, 8 good quality and 6 a neutral tasteless fish. The rejection level is 4. Using the scale in this way, the graph becomes S-shaped, indicating a rapid degradation of the fish Phase 1, a slower rate in Phases 2 and 3, and finally a high rate when the fish is spoiled. Other scales can be used and can change the shape of the graph. However, it is important to understand the kind of results desired from the sensory analysis in order to ask the right questions to the sensory assessors.

Although most sensory characteristics can only be measured meaningfully by humans, advances are being made in the development of instruments that can measure changes in individual quality attributes.

⁹ Available at: www.qim-eurofish.com



Source: Huss (1976).

Such instruments include the Instron and Bohlin Rheometer for measuring texture and other rheological properties. Microscopic methods combined with image analysis are used to assess structural changes, and “the artificial nose” is used to evaluate the odour profile (Nanto, Sokooshim and Kawai, 1993). A European project “Multi-sensor techniques for monitoring the quality of fish” has evaluated various physicochemical techniques: visible light spectrometry, electrical properties, image analysis, colour, electronic nose and texture (Nesvabda, 2003). Combining the outputs of the last three and calibrating with QIM sensory scores for appearance, smell and texture gives an artificial quality index, which is expected to provide accurate evaluation of fish freshness.

4.2 RESULTING POST-HARVEST LOSSES (HENRI LOREAL)

Post-harvest losses of fish occur in various forms. Physical fish loss is caused by poor handling and preservation, or the discarding of bycatch. Economic loss occurs when spoilage of wet fish results in a value decrease or when there is a need to reprocess cured fish, raising the cost of this operation. In addition, inadequate handling and processing methods can lead to nutritional loss. Similarly, the processing of large quantities of fish into animal feeds can be considered, under certain conditions, as a “loss” for human food security.

Although there are few verifiable estimates, post-harvest losses in small-scale fisheries can be very significant. As mentioned above, it has been estimated that the economic cost of losses in selected fisheries in selected countries in Africa ranges from US\$20 000 to US\$60 million – with quality issues accounting for up to 70 percent of the total losses (Akande and Diei-Ouadi, 2010). In Oman, a high incidence of post-harvest losses has been recognized as an economic problem affecting the development of the fisheries sector. The annual loss due to downgrading of fish has been estimated at nearly US\$65 million. The loss in quantity is about 10 percent for the entire traditional sector, which means a loss in potential revenue ranging from 12.5 to 20 percent (Linus, Al-Jufaili and Rahman, 2007).

Appropriate preservation methods can significantly reduce this loss, including from glut catches when the processing, distribution and marketing system cannot cope with

the large quantities of fish that are landed owing to seasonal or interannual variations in availability or abundance (Ababouch, 2003). Table 58 provides some examples from work in Africa of the causes of losses and potential interventions that can mitigate or reduce losses. As can be seen, in many cases, the interventions are simple (Akanke and Diei-Ouadi, 2010).

TABLE 58
Existing and potential loss reduction intervention initiatives

Physical loss	Causes or nature of losses	Existing intervention strategies	Where in use and by whom	Potential intervention strategies
Physical	Discarded trampled fish	Use of separation board on board canoes	United Republic of Tanzania, by lake sardine fishers	Redesigning of canoes
	Bird predation and pilferage	Use of camouflage to scare away the birds, and watchperson during sun-drying of the fish	United Republic of Tanzania, by sun-dried fish processors	Solar tent driers
	Fragmentation	Use of boxes instead of baskets	Ghana, by sardinella fish smokers	Packaging in sturdy wooden container
	Net entanglement in rocky areas	Indigenous knowledge of fishing area	Ghanaian fishers	Use echo sounder
Quality	Deterioration	Use of ice	Ghana and Kenya, by fishers and fish traders	Introduction of customized insulated boxes
	Insect infestation	Brining of fish before drying or smoking	Ghana, Mali and United Republic of Tanzania, by processors of smoked fish	Use of pirimiphosmethyl (Actellic ND) and other recognized natural and synthetic insecticides
	Rancidity and colour change	Reduce storage period	United Republic of Tanzania, in lake sardine sun-drying	Immersion in antioxidants
	Poor drying	Drying on bare floor or in some cases racks	Uganda and United Republic of Tanzania, by lake sardine processors	Use of mechanical driers. Smoke drying option or Brazilian salt-pressing technology
	Light and carbide fishing	Regulations on obnoxious methods of fishing	Ghana, by some fishers	Enforcement of fishing regulations against obnoxious methods of fishing

Source: Akanke and Diei-Ouadi (2010).

4.3 PRODUCT AUTHENTICITY (HENRI LOREAL)

Seafood companies and exporting countries are increasingly seeking to sell their products using commercial names of established international repute in order to derive maximum value and recognition. Although value-enhancing denominations are being sought for many species, they remain relatively rare.

The matter is exacerbated by the fact that different species may have the same common name in different countries (or even regions of the same country). On the other hand, the same species sometimes has different names in the same language in different locations within the same country. This may be a source of misleading information.



Market names such as “seabass” or “catfish” are frequently used in international trade, but they refer to very different species from various families.

In France, *D. labrax* is a high-value fish commercialized as “loup” or “bar”. *Anarhicas lupus* is a lower-value fish commercialized as “loup de l’Atlantique”. Other countries have different names for these species (Figure 29). Whole products cannot be confused but it may happen that an *A. lupus* fillet or fillet portion is sold as “filet de loup”.

The same observation applies to the name “catfish”, which is used for numerous species from very different fish families in international trade. The *Pangasius bocourti*

farmed in Viet Nam competes with other siluriforme species on the international catfish market.

Therefore, labelling provisions need to be sufficiently clear to prevent consumers from being misled and to prevent the creation of conditions of unfair competition in international trade. There is currently much interest in the issue as studies have shown that some 25–30 percent of fish on the market may be mislabelled (Jaquet and Pauly, 2008). Molecular genetic analysis has shown that some three-quarters of the fish sold in the United States of America as “red snapper” – the FDA’s legally designated common name for *Lutjanus campechanus* – are in fact another species, most commonly, rockfish (*Sebastes* spp.) (Marko *et al.*, 2004). Mislabelling to this extent not only defrauds consumers but could also adversely affect estimates of stock size if it influences the reporting of catch data that are used in fisheries management.

FIGURE 29 Local names for two fish species	
	
<i>Dicentrarchus labrax</i> (FIGIS Fact Sheet)	<i>Anarhichas lupus</i> (FIGIS Fact Sheet)
Local names: – United Kingdom: Bass / White salmon – France: Bar / Loup – Germany: Seabarsch / Seewolf	Local names: – United Kingdom: Atlantic wolffish / Cat-fish – France: Loup de l'Atlantique / Loup de mer – Germany: Katfisch / Seewolf

Since the mid-1960s, FAO has developed a programme to clarify and improve, on a national, regional and global scale, the identification of species of actual or potential interest to fisheries. This programme has produced world catalogues, regional identification sheets and national field guides, used for decades by many fish trading companies as the authoritative source of scientific and vernacular names and characteristics. In the last decade, information regarding bony fish and cartilaginous fish has progressively been included in FishBase. This work supports the deliberations of the Codex Committee on Fish and Fishery Products (CCFFP) on the identification of fish species for the standardization of fish and fishery products and on facilitating fish trade, especially exports from developing countries.

It is also important that scientific criteria be developed for listing species under a given denomination and a reliable methodology for verifying the authenticity of labelling claims.

Protein and DNA analyses are the most suitable methods for species identification of fillets or pieces of fish (Martinez, James and Loréal, 2005).

Other authentication issues with respect to the differentiation of wild and farmed fish, geographic origin and production methods require different analytical methods, such as trace element analysis or nuclear magnetic resonance techniques.

4.4 EXCESSIVE WATER UPTAKE (HENRI LOREAL)

Water is the main component of fish and seafood. Generally, consumers do not expect to purchase fresh or frozen fish containing artificially added water. Excessive water addition that brings about excessive fluid loss and shrinkage of the product when thawing and/or cooking is a deceptive practice that results in economic fraud and, often, the loss of quality and nutrients.

Water is used extensively during fish handling, preparation and processing. As a result, water uptake may occur. However, water is regularly found to be fraudulently added to seafood products, either directly or by addition of excessive amounts of waterholding compounds such as polyphosphates. Several studies have reported on the fraudulent increase of water content of scallops in Europe, and a recent report in the United Kingdom of Great Britain and Northern Ireland by Seafish has reviewed the use of polyphosphates as additives and also examined the testing methods for them in scallops and prawns (Seafish, 2012). At the international level, the CAC has initiated work on an international standard on quick-frozen scallop adductor muscle meat. The draft standard states that no added water is allowed, other than water that enters the flesh during normal processing (e.g. iced storage and washing operations). Consequently, it is necessary to establish the moisture content limit as a value that best describes the natural attribute of scallops, taking into account the variability in moisture content between species, and the effect of seasonality, harvest practices and other factors. This moisture level could be practically applied as a basis for determining whether excessive moisture has been added to the product.

Parallel to the standard, in 2012, the CCFFP discussed the drafting of a specific section of the Code of Practice for Fish and Fishery Products on the Processing of Scallop Meat. This includes an example of a flow diagram, identification of hazards and defects, and technical guidance to control, among other issues, added water.

5. Climate change and probable impact on fish safety

5.1 CLIMATE CHANGE AND THE MICROBIOLOGICAL SAFETY OF FISHERY PRODUCTS (MARK TAMPLIN AND IDDYA KARUNASAGAR)

5.5.1 Introduction

Scientists express little doubt that the earth's climate has changed since the start of the Anthropocene (Karl, 2007). In this period, variations have been observed for the cryosphere, in coastal, marine, freshwater and terrestrial systems, as well as in the frequency of severe weather events (IPCC, 2007a). In particular, change has been evident in freshwater and marine systems, where variations in surface seawater temperature, pH and sea level have been linked to events such as coral bleaching, harmful algal blooms, fish kills, and oyster disease, as well as outbreaks of human disease from known and emerging pathogens.

The debate about whether human activity is primarily responsible for climate change will continue because extended time intervals must be observed in order to conclude that a specific cause results in a specific effect. As such, this uncertainty delays implementation of risk management strategies that may need to be initiated in shorter time intervals. Whether anthropomorphic or natural, the earth's climate is changing, and such events may influence the safety (positive and negative) of food harvested from marine and freshwater environments.

Seawater temperature, level, salinity and pH have changed in the past century (IPCC, 2007b; Karl, 2007; Levitus *et al.*, 2000). On average, the ocean has increased one-third of a degree Celsius in the last 50 years (Levitus *et al.*, 2000). In addition, the sea level has risen at a global average rate of 1.7–1.8 mm per year in the last century, and at an elevated rate of 3 mm per year in the past 10 years (IPCC, 2007c).

The average pH of the ocean, 8.4, has dropped by approximately 0.1 units since pre-industrial times (Kintisch and Stokstad, 2008). This has been caused, in part, by the conversion of carbon dioxide and other atmospheric gases to water-soluble acidic compounds, such as carbonic, sulphonic and nitric acids. By 2100, some estimates indicate that ocean pH will drop another 0.4 units at the current rate of carbon increase. In addition to the direct effect of pH on biota, increased temperature can also compound the negative effects of pH on marine life (O'Donnell, Hammond and Hofmann, 2009).

Ocean warming, sea-level rise, and changes in ocean chemistry are driven, in part, by increases in atmospheric greenhouse gases (Kite-Powell *et al.*, 2008). Carbon dioxide, although relatively low in concentration compared with other gases, absorbs energy and contributes to global warming. Atmospheric gases can also exert a direct effect on microbial physiology as well as through compounds that are formed when gases dissolve in seawater.

It is well established in the field of microbial ecology that the environment selects for types and levels of bacteria that live in a particular habitat. Baas-Becking (1934) stated that "Everything is everywhere, but the environment selects." Although not every environmental parameter exerts as strong an influence on microbial viability as temperature, water activity (salinity) and pH do. Importantly, these same factors are also markedly affected by climate change.

Although changes in marine microbial communities do not always translate into changes in risk to animal and human health, it is important to understand how and to what degree variations in environmental parameters can influence microbial community structure, especially those that impact health. Such knowledge will provide insights into potential risk management strategies.

5.1.2 Climate change and probable impact on fisheries

Climate-mediated changes in environmental conditions cascade through an ecosystem, initially affecting the most susceptible species and producing a change in the balance of the community. An overview of the current scientific knowledge on climate change and its implication for fisheries and aquaculture was provided by an FAO Expert Workshop held in 2008 (Cochrane *et al.*, 2009). There was agreement that climate change is affecting the seasonality of biological processes, altering marine and freshwater food webs with unpredictable consequences for fish production.

Climate variability and human activities may change host resistance (positive or negative) and facilitate pathogen transmission through exposure of a new host to a pathogen, or vice versa. In addition to a direct effect, environmental change can also affect the physiology and ecology of other marine biota in the same habitat. This includes diseases that may affect animal health and also influence the balance in host-microbe interactions (e.g. mutualistic, synergistic, symbiotic, parasitic and commensal).

The most sensitive aquatic species are those that exist in habitats where environmental parameters are closer to their physiological limit, such as in tropical environments (e.g. corals), but which are further away from the physiological limit of pathogens. Such species can experience greater shifts in pathogen loads (Porter *et al.*, 1989). Higher susceptibility would also be likely to occur for those species with complex tissue systems, followed by multicellular organisms, and then single-cell organisms.

Severe bleaching of corals caused by the loss of algal symbionts and or algal pigments has been reported in periods when sea surface temperatures exceed summer maxima by 1–2 °C for a few weeks (Glynn, 1996), and bleached corals are vulnerable to pathogenic micro-organisms (Brandt and McManus, 2009). Studies have shown that a daily average increase of 1 °C during summer months results in a shift from *Spongiobacteria*-related species to predominantly *Vibrio* spp. (Bourne *et al.*, 2008). This shift occurs before the visible bleaching event whereby coral zooxanthellae substantially decrease. It has been suggested that large-scale bleaching and mortality could occur in coral reefs by 2050 (IPCC, 2007d).

Diseases in the marine environment can be caused by pollution, introduction of terrestrial organisms, harvesting and climate change. There is little systematic and conclusive evidence of a direct link between any of these factors. A detailed review by Lafferty, Porter and Ford (2004) describes diseases of multiple species of marine life, including corals, abalones and urchins. The northward spread of the MSX (multinucleated spore unknown) disease among *Crassostrea virginica* oysters, caused by the protozoan parasite *Haplosporidium nelsoni*, on the east coast of the United States of America has been attributed to climate warming (Hofmann *et al.*, 2001).

When considering transmissible diseases, different complexes may be responsible and potentially be affected by climate change (Shope, 1991). These include: two-factor complexes that involve the causative agent and host; a three-factor complex that also includes a vector; and a four-factor complex that involves an intermediate host. The latter two complexes would be likely to be more susceptible to climate change, as the vectors and intermediate host (or hosts) may be affected by environmental factors.

Harvell *et al.* (1999) provide a comprehensive review of emerging marine diseases reported from 1938 to 1997, and they describe possible links to climate change and anthropogenic factors. Examples of these mass mortalities (i.e. > 10 percent

mortality within populations) include seals, dolphins, pilchards, herrings, scallops, clams, abalones, oysters, urchins, corals, kelp, seagrasses, algae and sponges. While more-effective reporting may account for higher rates of mortality in recent years, climate change and human activity are believed to have caused physiological stresses, host and range shifts in pathogens, and global transport of species (Harvell *et al.*, 1999).

Records of the El Niño Southern Oscillation (ENSO) show that it has occurred at a frequency of one or two episodes per decade over the past 5 000 years. However, episodes have occurred more frequently and for longer durations since the 1970s (Rodbell *et al.*, 1998; Trenberth and Hoar, 1996). ENSO events have documented impacts on marine species, which include changes in bacterial communities leading to marine host infection (Kushmaro *et al.*, 1996).

Elevated surface seawater temperature has also been shown to influence the prevalence of Dermo disease of the eastern oyster, *Crassostrea virginica*, caused by the protozoan *Perkinsus marinus*. During El Niña events, drier weather leads to increase salinity in estuarine environments, favouring the survival of *P. marinus* (Kim and Powell, 1998). It is possible that increased oyster disease could reduce oyster host defences systems, potentially elevating the load (i.e. exposure levels) of pathogenic *Vibrio* spp.

More recently, large mortalities have been reported in shellfish hatcheries on the North American west coast; the reported cause was *Vibrio tubiashii* (Elston *et al.*, 2008). The death of larval and juvenile bivalves was associated with elevated levels of *V. tubiashii* in coastal waters. It was suggested that these mortalities were associated with the mixing of unusually warm surface seawater and upwelled seawater that was high in nutrients and *Vibrio* spp. (i.e. 1.6×10^5 cfu/ml). The disease occurred in three new hosts, Pacific (*Crassostrea gigas*) and Kumamoto (*C. sikamea*) oysters and geoduck clams (*Panope abrupta*) (Elston *et al.*, 2008).

5.1.3 Climate change and probable impact on fish safety

As stated by Karl “Microorganisms inhabit all marine ecosystems, from the tropics to the sea ice and from the well-lit surface waters to the deep abyss; they truly are the ‘unseen majority’” (Karl, 2007). Micro-organisms are involved in important transformations of nutrients, the acquisition and transduction of solar energy, the production and utilization of greenhouse gases (e.g. carbon dioxide, nitrous oxide and methane) and constitute a great pool of genetic diversity (Karl, 2007). Some species are also the aetiological agents of human disease.

Through the consumption of seafood, people are exposed to a variety of microbial species. The outcome of such exposure, depending on characteristics of the population and the type of exposure, can lead to negative (e.g. gastrointestinal illness and toxic poisoning) or positive health effects (e.g. nutritional and health benefits) (Kite-Powell *et al.*, 2008). Such health outcomes can be modelled and estimated through quantitative microbial risk assessment (QMRA) (Ross and McMeekin, 2009).

In places where pathogens are controlled through sanitary infrastructure but a reservoir exists, changes in climatic conditions could result in the expansion of populations of pathogens to levels that may be more difficult to control through hygienic practices. Such a situation has been described for *V. cholerae* in the United States Gulf of Mexico (Shope, 1991; Blake *et al.*, 1980) and in Peru (Tauxe, Mintz and Quick, 1995), where pathogenic strains unexpectedly reached concentrations that led to human outbreaks and epidemics of cholera.

Climate change is expected to accelerate the water cycle, with increased precipitation in the tropics and at high altitudes, drier conditions in the subtropics and increased frequencies of extreme droughts and floods (IPCC, 2007e). Events such as floods are likely to disrupt sanitary infrastructure around fish harvesting/aquaculture sites, affecting fish safety. Outbreaks of cholera during floods in Bangladesh have

been documented (Schwartz *et al.*, 2006) and although cholera transmission may be mainly through water, contamination of seafood also needs to be considered. During the cholera outbreak in Peru, fish in domestic markets were contaminated with *V. cholerae* O1 (FAO/WHO, 2005b). In many parts of the world, oysters are consumed raw and consumer safety is managed by monitoring the growing waters or shellfish for the presence of faecal coliforms. Extreme weather events such as floods and hurricanes may lead to a breakdown in the sanitary infrastructure and dissemination of pathogenic micro-organisms. The presence of *Salmonella* in rivers and the marine environment has been related to torrential rains and storm-generated flows (Martinez-Urtaza *et al.*, 2004a), and the pathogen could then reach aquaculture sites or contaminate fish in coastal waters.

The viability of micro-organisms is strongly influenced by temperature, pH and water activity. These factors affect the structure of water surrounding important macromolecules and, thereby, affect processes involved in cellular maintenance, growth and death (Ratkowsky, Olley and Ross, 2005). Variations in these key environmental factors can have different effects, depending on the bacterial species and the physiological state of the cell, by increasing or decreasing bacterial growth and inactivation rates (Tamplin, 2009).

5.1.3.1 Effects of temperature and pH

As a general rule, for a 10 °C increase in temperature, the rate of reaction (i.e. bacterial growth) doubles or triples (Beavon, 2010). This rule of thumb derives from the Arrhenius equation. The change is more pronounced the further away from the optimum (for growth rate). For example, mathematical models for *E. coli* growth rate (e.g. Presser, Ratkowsky and Ross, 1997), indicate that the rate of growth increases approximately 12-fold from 8 °C to 18 °C.

The scientific literature and numerous predictive models describe in detail how temperature affects the growth of bacteria, including pathogenic and non-pathogenic species (Tamplin, 2009; Tamplin, Baranyi and Paoli, 2003; McMeekin, Olley and Ross, 1993). As such, tools are available to estimate the effects of temperature change on the growth of a number of seafood-borne bacterial pathogens. However, most of these models were developed for bacterial growth in pure culture systems under non-limiting nutrient conditions. Therefore, the models are likely to overestimate the growth of bacteria in marine and freshwater environments where nutrients are limited and where they exist in a complex milieu of competing micro-organisms. In the latter case, one would expect that competition among bacterial strains and species would permit faster growth of some species than others (i.e. the Jameson effect; Ross, Dalgaard and Tienungoon, 2000; Stephens *et al.*, 1997). Nevertheless, predictive models provide a good foundation upon which to understand how bacterial species will respond to environmental change and resulting human exposure levels in fishery products.

The effect of temperature variation on aquatic reservoirs of bacterial pathogens has received little attention in comparison with effects on more complex marine biota. Various reports support the hypothesis that an increase in seawater temperature would lead to increased levels of bacterial pathogens in seafood (i.e. higher human exposure) (FAO/WHO, 2003b; 2005c; FDA, 2005). This assumption is also supported by a study of terrestrial food-borne illness in England and Wales, the United Kingdom of Great Britain and Northern Ireland, from 1974 to 2006, in which Lake *et al.* (2009) showed that campylobacteriosis, salmonellosis, *Salmonella* Typhimurium and *Salmonella* Enteritidis were associated with elevated air temperature during and prior to the reported cases.

Human disease caused by *Vibrio* spp. shows a strong relationship to seawater temperature. This is reflected in the seasonality, as well as unexpected outbreaks, of illnesses caused by *Vibrio vulnificus*, *Vibrio cholerae* and *Vibrio parahaemolyticus*

(FAO/WHO, 2003b; FAO/WHO, 2005c; Ford *et al.*, 2009). For example, the 1991 epidemic of *V. cholerae* in Latin America and the outbreak of shellfish-associated *V. parahaemolyticus* disease in Chile in 2004 are believed to have been influenced by ENSO events (Harth *et al.*, 2009; Pascal *et al.*, 2000). In addition, Paz *et al.* (2007) showed that *Vibrio vulnificus* disease (bacteremia and wound infection) among fish market workers in Israel appears to have paralleled marked increases in mean ambient air temperature. Temperature is also expected to cause a poleward expansion of ciguatera poisoning, by shifting the distribution of marine algae that produce ciguatoxins (IPCC, 2007b).

Temperature may also affect uptake of chemical contaminants from the environment by fish and shellfish. Fish safety managers are particularly concerned about levels of mercury, cadmium and lead in fish, and there are regulatory levels prescribed for these. Methylation of mercury has been shown to be affected by temperature, and the uptake of methylmercury has been found to increase by 3–5 percent for each 1 °C rise in water temperature (Booth and Zeller, 2005). Gaden *et al.* (2009) reported that mercury levels in Canadian Arctic ringed seals and cod are linked to vanishing sea ice caused by global warming.

As with temperature, there is a wealth of information about the quantitative effect of pH on bacteria, but less so for those that reside in aquatic environments. In the environments in which humans produce fish, the pH ranges from being close to neutral in freshwater to being more basic in marine and brackish waters.

A change in pH can move bacteria closer to or further away from the optimum growth rate, depending on the species. Considering this and the complexity of microbial communities in aquatic environments, it will probably be difficult to accurately predict shifts in populations at the species level. However, pathogenic strains are a much smaller subset of the community, and predictive models will probably assist in estimating such effects.

5.1.3.2 Effects on sea level

Additional effects of climate change include spatial variation in precipitation, severe weather events and glacial melting. Globally, in the past ten years, these events appear to be elevating sea levels at a rate of 3 mm per year (IPCC, 2007c). Elevated sea levels and periodic floods expand habitats available for fish and shellfish (IPCC, 2007f). This affects marine and freshwater fisheries and aquaculture in fundamental ways. For example, a reduction in salinity significantly affects the physiology of fish, causing shifts in habitats and migration. This particularly affects aquaculture operations in marine and brackish environments. Conversely, some geographical areas may experience abnormally lower rainfall, affecting inland fisheries and freshwater aquaculture.

Excessive rainfall and elevated sea levels also introduce new and increased numbers of micro-organisms into estuaries and freshwater bodies. This has effects on microbial ecology and on the prevalence and levels of pathogens that affect fish species and humans (IPCC, 2001). For example, in Canada, outbreaks of *Escherichia coli* O157:H7 occurred when rainfall and flooding washed faecal material from neighbouring cattle operations into municipal wells (Hrudey *et al.*, 2003).

5.1.3.3 Emergence of pathogens

There is an undiscovered diversity of micro-organisms in aquatic environments. This fact makes the prediction of what, where, when and how a new pathogen will emerge very uncertain (Venter *et al.*, 2004). More probably, risk assessors and risk managers will rely on precedent and a few predictive models to forecast more accurately the incidence and levels of recognized pathogens (e.g. *Vibrio* spp.).

“Emergence” has gained a more specific definition in public health terms, signifying an increase in prevalence, a new species or strain variety, infections appearing in new

host populations and recognized infections spreading by a new transmission route (Woolhouse and Gaunt, 2007). It can be expected that higher water temperature will result in increased concentrations of bacteria, a larger pool of micro-organisms with mutations, and greater probability of gene transfer. This will probably be influenced by the movements of and shifts in populations of aquatic life and associated microbial communities.

The emergence of *V. cholerae* serotype O1 in the coastal waters of Peru, and the resulting epidemic in other Latin American countries over an 18-month period, is a more recent example. The resulting impact included 1 million cases of cholera and 10 000 reported deaths (Tauxe, Mintz and Quick, 1995). Similarly, *V. cholerae* O139 emerged as a new serotype in India (Faruque, Albert and Mekalanos, 1998). This change in *V. cholerae* capsular polysaccharide posed a great threat, as protective immunity to cholera is largely O-antigen specific. The factors influencing this were complex, with a mix of public health infrastructure, bacterial mutation, and coincidences in human behaviour (Ford *et al.*, 2009; Sumilo *et al.*, 2007).

In the Chilean *V. parahaemolyticus* outbreak, strains associated with human illness in 2004 were the pandemic serotype O3:K6. However, since 2007, other strains have emerged and have been associated with human disease. Evidence indicates that genes encoding capsular exopolysaccharide and virulence may have been horizontally transferred among other *V. parahaemolyticus* strains, producing strains with an enhanced ability to survive in the environment but being less infectious to humans (Nair *et al.*, 2007).

The O3:H6 serotype was first reported in Calcutta (Kolkata), India, in 1996 (Okuda *et al.*, 1997). The clone spread throughout most Southeast Asian countries from 1996 to 1997 (Martinez-Urtaza *et al.*, 2008). In 1997, it was reported at a single location outside Asia, i.e. Chile (Gonzalez-Escalona *et al.*, 2005). These authors suggest that the arrival of the Asian O3:K6 serotype was facilitated by warm equatorial water displaced from Asia to America by the most recent two El Niño episodes. In addition, through a very thorough analysis of sea surface temperature and multivariate ENSO index data, they show a strong association between these environmental parameters and the incidence of *V. parahaemolyticus* infections. The arrival of the O3:K6 strains corresponded closely with the propagation of the 1997 El Niño event.

Epidemiologists normally recognize an emerging pathogen only when its incidence reaches some threshold among other infectious diseases in a population, produces very unique symptoms, or affects a unique human subpopulation. Therefore, it can be assumed that new pathogens are continuously emerging and that many may not be recognized and disappear. Whether they persist depends on their ability to survive in a new environment and within the susceptible population. It is also important to consider that, while emerging infectious diseases are undesirable for humans, the aetiological species may have important roles in the marine environment in promoting biodiversity (Lafferty, 2003).

5.1.3.4 Other considerations

Changes in atmospheric gases as a result of climate change can also affect global fisheries and transmission of infectious diseases. For example, oxygen levels decrease in warmer seawater (Lafferty, Porter and Ford, 2004). Other studies indicate that undersaturation of aragonite, the more soluble form of calcium carbonate, will occur in the Southern Ocean by 2050 when the atmospheric CO₂ concentration reaches 450 ppm (McNeil and Matear, 2008). Among other factors, this will affect the formation of marine exoskeletons and potentially increase the risk of infection.

The rate of disease transfer can also be affected (positively and negatively) based on factors that influence densities of fish, such as through harvesting pressure (Jackson *et al.*, 2001). Smaller populations of fish can reduce pathogen transmission rates and

thereby limit the density of the pathogen. Conversely, higher fish densities can increase pathogen load and the probability of exposure.

5.1.4 Mitigation of the probable impact on fish safety

There is much less published research about the ecology of pathogenic bacteria in fishery environments compared with research on terrestrial animals and foods. As such, funding organizations should consider investing in research to fill key knowledge gaps that could identify ways to protect public health.

Risk assessment may be the best strategy for managing, or at least preparing for, the potential impact of climate change on fish and human safety. In this regard, a number of risk assessments have been conducted by FAO and WHO in separate and joint consultations, as well as by other public health bodies (EFSA, 2008; FAO, 2005b; FAO/WHO, 2003, 2004a, 2005b, 2005c, 2006b; FDA, 2005; Ivanek *et al.*, 2004; Wong *et al.*, 2006). Although significant efforts in themselves, these assessments have mostly focused on *Vibrio* spp. and *Listeria monocytogenes*.

It is recognized that the full benefits of risk assessment have not been realized. Much more attention needs to be given to translating QMRA into tools that are simple to use and have practical applications at the national, regional and local levels. In this regard, FAO/WHO has prepared six case studies of pathogen-food combination, two of which are *V. vulnificus* in oysters and *L. monocytogenes* in smoked fish (FAO/WHO, 2006b). The scenarios illustrate how QMRA can be used to identify “sensitive” points in the production–supply chain, where risk management strategies can significantly influence risk.

Likewise, QMRA could be used to identify environmental conditions that are sensitive to the effects of climate change, and then identify potential mitigation strategies to lower risk. Strategies to forecast risk also need to consider the parallel development of better public health measures and engineering interventions.

Such an approach could then be used to determine ways to implement remote monitoring of environmental parameters known to influence the viability of pathogens, as well as conditions that promote the emergence of new strains, such as via gene transfer or high mutation rates. Without such underlying models, remote sensing may offer far less value as an effective warning system.

5.1.5 Conclusions

More research is needed in order to understand the implications of climate variation on the productivity of fisheries and human health. However, based on current evidence and predictive models, it can be assumed that climate change will produce effects, although the precise outcomes remain highly uncertain. Karl (2007) has considered such scenarios and states that “These case studies reveal the importance of, and the need for, comprehensive analyses – ranging from genomes to biomes, coupled to interdisciplinary physical and chemical observations of broad temporal–spatial scales – before a comprehensive understanding of the role of micro-organisms in oceanic ecosystems can be achieved.”

For commercial fisheries, climate change, especially increased temperature, will probably result in a higher incidence of fish mortality. For fish processors, one can anticipate greater costs associated with food safety and sanitation processes to control contamination at the processing level, and subsequent product handling at wholesale level, retail level and by the consumer.

5.2 IMPACTS OF CLIMATE CHANGE ON HARMFUL ALGAL BLOOMS AND SEAFOOD SAFETY (GUSTAAF HALLEGRAEFF)

5.2.1 Introduction

In a strict sense, harmful algal blooms are completely natural phenomena that have occurred throughout recorded history. However, even non-toxic algal blooms can have devastating impacts when they lead to kills of fish and invertebrates by generating anoxic conditions in sheltered bays. Other algal species, although non-toxic to humans, can produce exudates that can cause damage to the delicate gill tissues of fish (raphidophytes *Chattonella*, *Heterosigma*, and dinoflagellates *Karenia*, *Karlodinium*). Whereas wild fish stocks are free to swim away from problem areas, caged fish in intensive aquaculture operations are trapped and, thus, can suffer devastating mortalities. Of greatest concern to human society are algal species that produce potent neurotoxins that can find their way through shellfish and fish to human consumers where they evoke a variety of gastrointestinal and neurological illnesses. One of the first recorded fatal cases of food poisoning after eating contaminated shellfish happened in 1793, when Captain George Vancouver and his crew landed in British Columbia (Canada) in an area now known as Poison Cove. He noted that, for local Indian tribes, it was taboo to eat shellfish when the seawater became bioluminescent due to algal blooms by the local dinoflagellate *Alexandrium catenella/tamarense*, which is now known to be a causative organism of PSP. The increase in shellfish farming worldwide is leading to more reports of PSP, DSP (first documented in 1976 in Japan), NSP (reported from the Gulf of Mexico as early as 1844) and ASP (first identified in 1987 in Canada). The explorer Captain James Cook already suffered from the tropical illness of CFP from fish when visiting New Caledonia in 1774. Worldwide, almost 2 000 cases of food poisoning from consumption of contaminated fish or shellfish are reported each year. Some 15 percent of these cases prove fatal. If not controlled, the economic damage through the slump in local consumption and exports of seafood products can be considerable. Whales and porpoises can also become victims when they receive toxins through the food chain via contaminated zooplankton or fish. In the United States of America, poisonings of manatees in Florida via seagrasses and, in California, of pelicans and sea lions via contaminated anchovies have also been reported (Hallegraeff, Anderson and Cembella, 2003).

In the past three decades, harmful algal blooms seem to have become more frequent, more intense and more widespread. Four explanations for this apparent increase in algal blooms have been proposed: (i) a greater scientific awareness of toxic species; (ii) the growing utilization of coastal waters for aquaculture; (iii) the stimulation of plankton blooms by domestic, industrial and agricultural wastes and/or unusual climate conditions; and (iv) the transportation of algal cysts either in ships' ballast water or associated with moving shellfish stocks from one area to another (Hallegraeff, 1993).

Few long-term records exist of algal blooms at any single locality; ideally, at least 30 consecutive years of data would be needed. Therefore, whether or not the apparent global increase in harmful algal blooms represents a real increase is a question that will probably not be answered conclusively for some time to come.

The growing interest in using coastal waters for aquaculture is leading to a greater awareness of toxic algal species. People responsible for deciding quotas for pollutant loadings of coastal waters, or for managing agriculture and deforestation, should be made aware that one probable outcome of allowing polluting chemicals to seep into the environment will be an increase in harmful algal blooms. In countries that pride themselves on having disease- and pollution-free aquaculture, every effort should be made to quarantine sensitive aquaculture areas against the unintentional introduction of non-indigenous harmful algal species. Nor can any aquaculture industry afford not to monitor for an increasing number of harmful algal species in water and for an

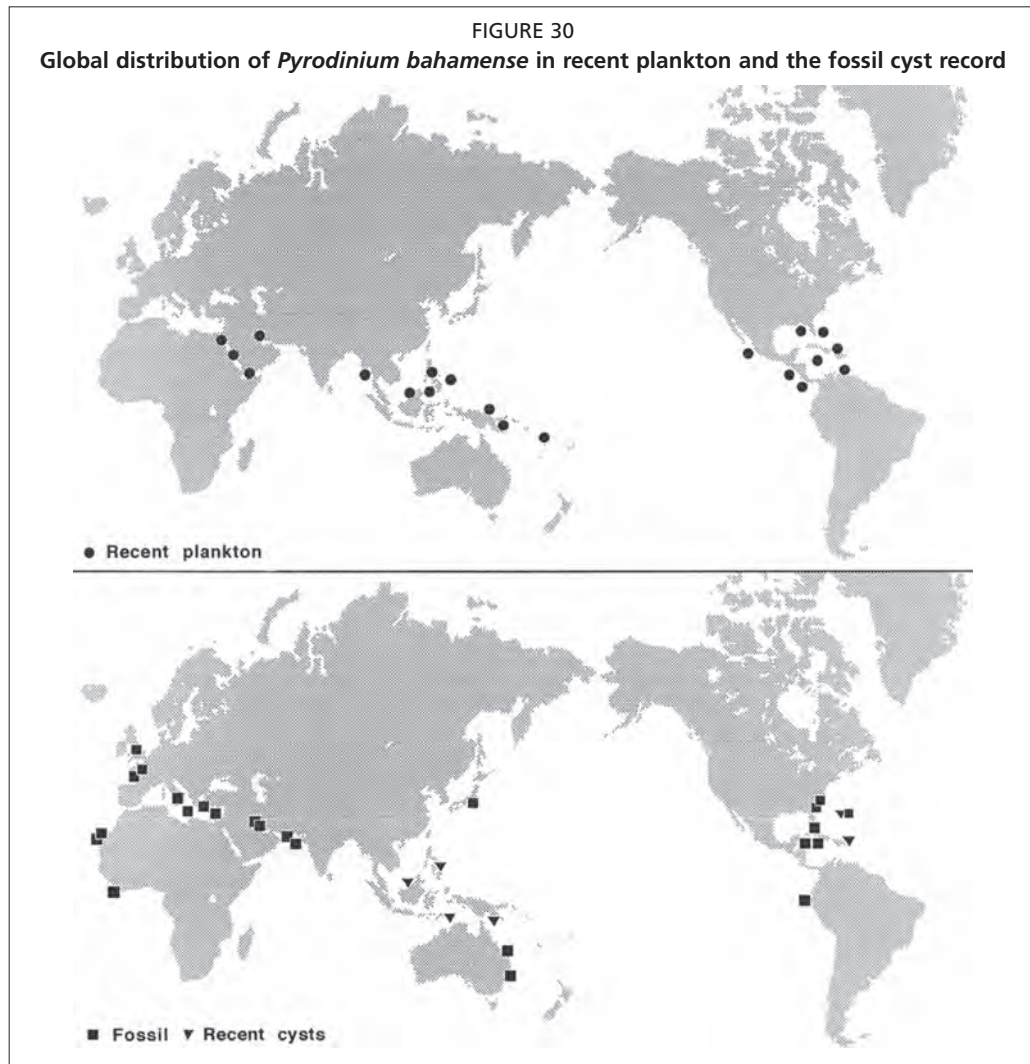
increasing number of algal toxins in seafood products – using increasingly sophisticated analytical techniques such as LC-MS (see Section 3.2.5). Last, global climate change is adding a new level of uncertainty to many seafood safety monitoring programmes, as are range extensions of harmful algal bloom species through their being transported in ships' ballast water and as a consequence of increases in sea surface temperatures (Hallegraeff, 2010).

5.2.2 Range extensions by transport in ships' ballast water

Ballast water is seawater that has been pumped into a ship's hold or dedicated ballast tanks to steady it by making it heavier and thus less likely to roll; the water is released when a ship enters port. Ballast water on cargo vessels was first suggested as a means of dispersing marine plankton more than 100 years ago (Ostenfeld, 1908). However, it was only in the 1980s that the problem sparked considerable interest, after evidence was brought forward that non-indigenous toxic species such as the PSP dinoflagellate *Gymnodinium catenatum* had been introduced into sensitive aquaculture areas of Australian waters, with disastrous consequences for commercial shellfish farms (McMinn *et al.*, 1997). Similarly, the PSP dinoflagellate *Alexandrium catenella*, of a diagnostic temperate Asian ribotype, has appeared on French and Spanish Mediterranean coasts in the past two decades (Lilly *et al.*, 2002). Ecosystems disturbed by pollution or climate change are more prone to ballast water invasions (Stachowicz *et al.*, 2002).

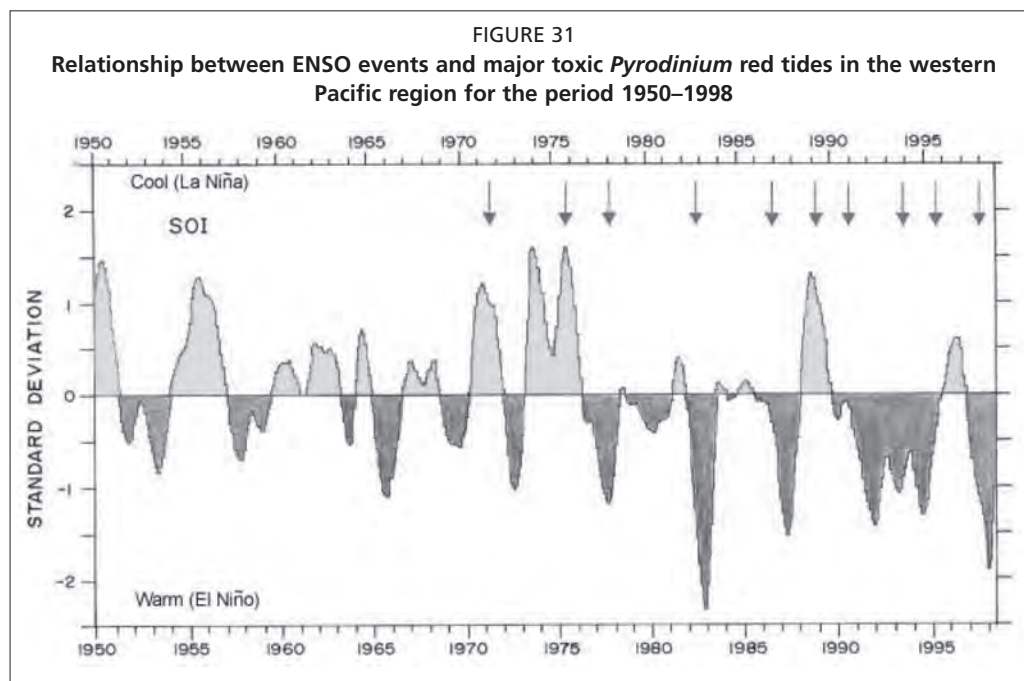
5.2.3 Algal bloom range extensions and climate change

The dinoflagellate *Pyrodinium bahamense* is currently confined to tropical, mangrove-fringed coastal waters of the Atlantic and Indo-West Pacific. A survey of cyst fossils (named *Polysphaeridium zoharyi*) going back to the warmer Eocene 50 million years ago indicates a much wider range of distribution in the past. For example, in the Australasian region at present, the alga is not found farther south than Papua New Guinea, but, some 100 000 years ago, the alga ranged as far south as what is now Sydney Harbour, Australia (McMinn, 1989). There is concern that, with an increased greenhouse effect and warming of the oceans, this species may return to Australian waters (Figure 30). In the tropical Atlantic, in areas such as Bahia Fosforescente in Puerto Rico and Oyster Bay in Jamaica, the glowing red-brown blooms of *Pyrodinium* are a major tourist attraction. At first considered harmless, *Pyrodinium* blooms gained a more sinister reputation in 1972 in Papua New Guinea after red-brown water discolorations coincided with the fatal food poisoning (diagnosed as PSP) of three children in a seaside village. Since then, these toxic blooms have apparently spread to Brunei Darussalam and Sabah, Malaysia, (1976), the central (1983) and northern Philippines (1987) and North Maluku, Indonesia. There is strong circumstantial evidence of a coincidence between *Pyrodinium* blooms and weather perturbations linked to the ENSO (Figure 31). *Pyrodinium* is thus a serious public health and economic problem for these tropical countries, all of which depend heavily on seafood for protein. In the Philippines alone, *Pyrodinium* has now been responsible for more than 2 000 human illnesses and 100 deaths resulting from the consumption of contaminated shellfish, sardines and anchovies (Hallegraeff and MacLean, 1989; Azanza and Taylor, 2001). Erickson and Nishitani (1985) reported exceptional PSP episodes by *Alexandrium tamarense/catenella* in the Pacific Northwest during 7 out of 9 ENSO events between 1941 and 1984.



Source: Hallegraeff (1993).

Until recently, NSP by the dinoflagellate *Karenia brevis* was considered to be endemic to the Gulf of Mexico and the east coast of Florida, the United States of America, where red tides had been reported as early as 1844. An unusual feature of NSP is the formation by wave action of toxic aerosols, which can lead to respiratory asthma-like symptoms in humans. In 1987, a major Florida bloom was dispersed by the Gulf Stream northward into the waters of North Carolina, the United States of America, where it has since persisted (Tester *et al.*, 1991; Tester, Geesey and Vukovich, 1993). In early 1993, more than 180 human NSPs were reported from New Zealand. Most likely, this mixed bloom of *Karenia mikimotoi* and related species was again triggered by the unusual weather conditions at the time, including higher than usual rainfall and lower than usual temperature, which coincided with an El Niño event (Chang *et al.*, 1998; Rhodes *et al.*, 1993).

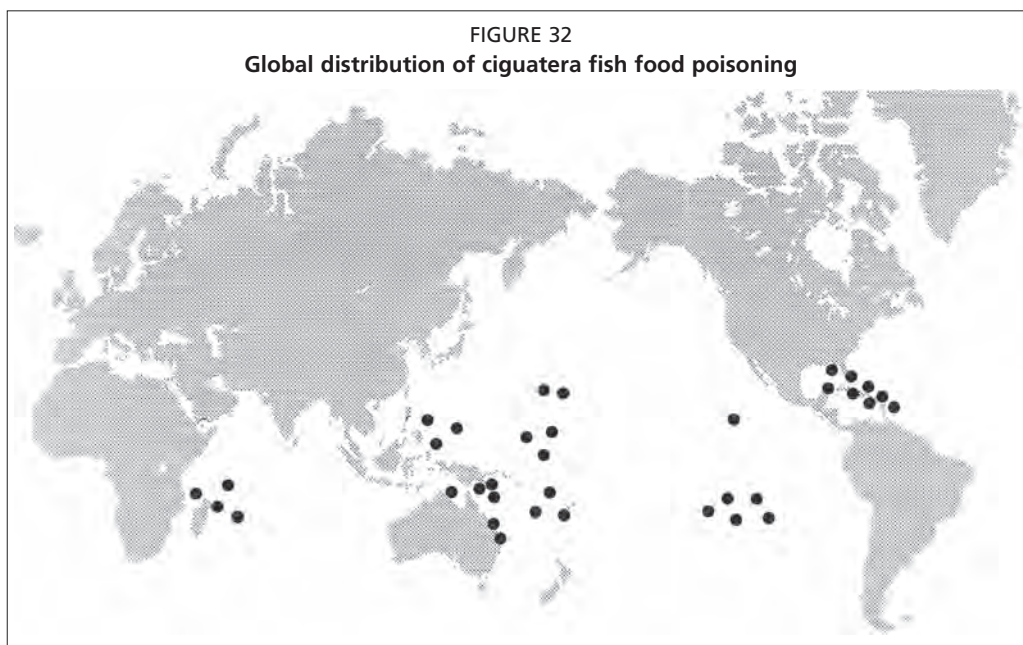


Notes: The Southern Oscillation Index (SOI) values are based on readings taken in Tahiti (French Polynesia), Hawaii (the United States of America) and Darwin (Australia). Arrows indicate years when *P. bahamense* red tides occurred in the Philippines and Malaysia.

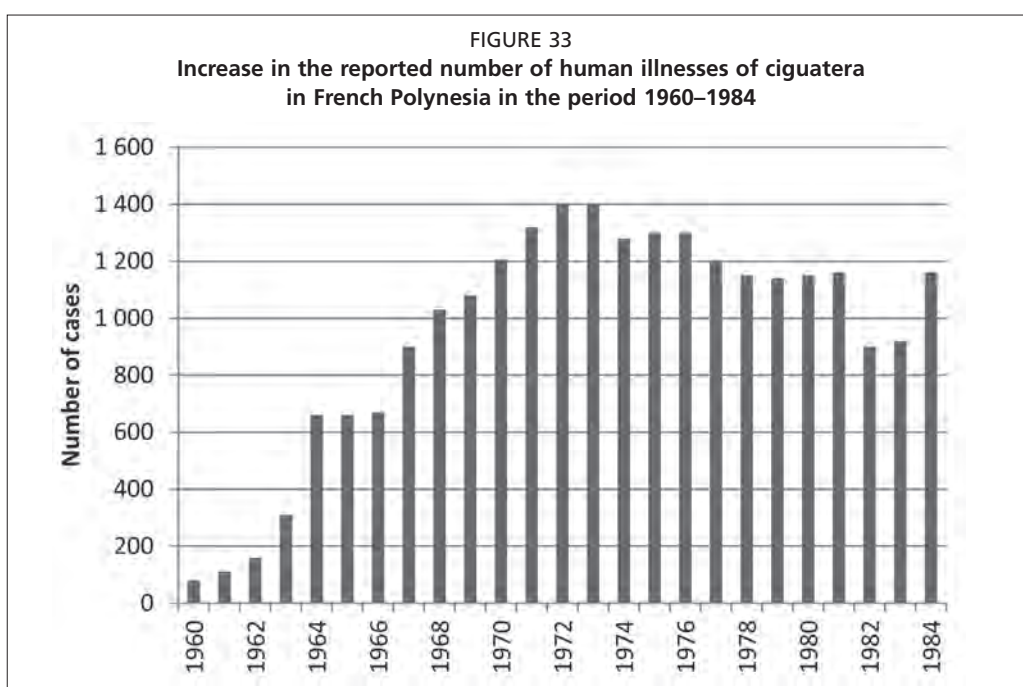
Source: Azanza and Taylor (2001).

Ciguatera caused by the benthic dinoflagellate *Gambierdiscus toxicus* is a tropical food poisoning syndrome well-known in coral reef areas in the Caribbean, Australia and, in particular, French Polynesia (Figure 32). Whereas, in a strict sense, this is a completely natural phenomenon, from being a rare disease two centuries ago, ciguatera has now reached epidemic proportions in French Polynesia. From 1960 to 1984, more than 24 000 patients were reported from this area, which is more than six times the average for the Pacific as a whole. Evidence is accumulating that reef disturbance by hurricanes, military activities and tourist developments (Bagnis, Bennett and Barsinas, 1985), as well as coral bleaching (linked to global warming) and perhaps, in future, increasing coral damage due to ocean acidification (Hoegh-Guldberg, 1999) are increasing the risk of ciguatera (Figure 33). Ciguatera dinoflagellates are predicted to become one of the winners from climate change (Tester *et al.*, 2010).

In the Australian region, *Gambierdiscus* dinoflagellates are well-known from the tropical Great Barrier Reef and southwards down to just north of Brisbane. However, in the past five years, this species has exhibited an apparent range extension into southeast Australian seagrass beds as far south as Melbourne, aided by a strengthening of the East Australian Current. In the same region, the red-tide dinoflagellate *Noctiluca scintillans* (known from the Sydney region as early as 1860) has, since 1994, expanded its range into southern Tasmanian waters, where it has caused problems for the salmonid fish farm industry (McLeod *et al.*, 2012). In the North Sea, an analogous northward shift of warm-water phytoplankton has occurred as a result of regional climate warming (Hays, Richardson and Robinson, 2005; Edwards and Richardson, 2004; Richardson and Schoeman, 2004).



Source: Hallegraeff (1993).



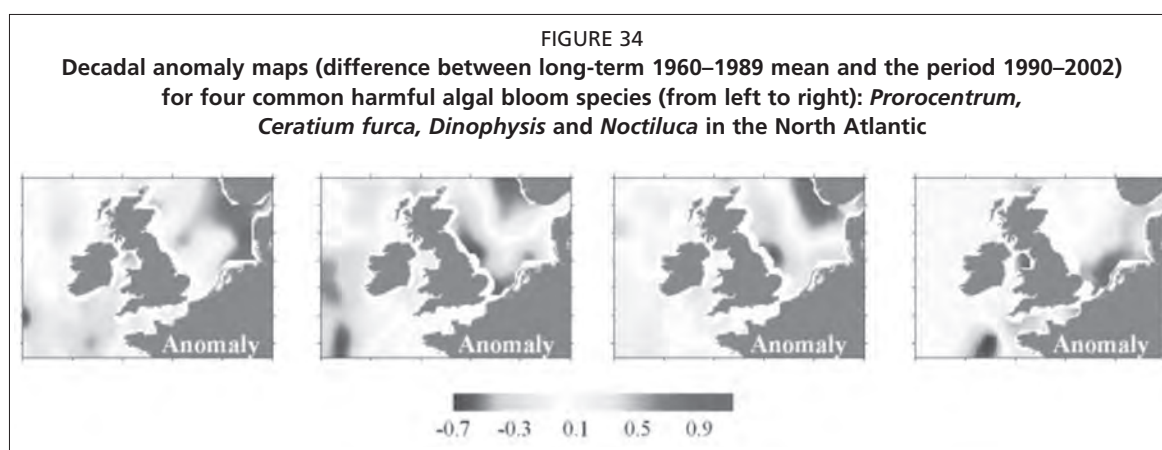
Note: Increased reef damage by coral bleaching or ocean acidification is predicted to stimulate ciguatera.
Source: Bagnis, Bennett and Barsinas (1985).

5.2.4 Predicted impact of climate change on phytoplankton abundance

Phytoplankton play a central role in several global biogeochemical cycles. Through the process of photosynthesis, they are also a major consumer of carbon dioxide. The ability of the oceans to act as a sink for anthropogenic carbon dioxide largely relies on the conversion of this gas by phytoplankton into particulate organic matter, and subsequent partial loss to the deep ocean (the so-called “biological pump”). Phytoplankton also have important feedback effects on climate. Some species (e.g. *Phaeocystis*) are producers of dimethylsulphonium propionate, a precursor of dimethylsulphoxide, which in the atmosphere is oxidized into sulphate, which forms

condensation nuclei for clouds (Charleson *et al.*, 1987). Therefore, phytoplankton can indirectly affect albedo and precipitation and, hence, coastal runoff, salinity, water column stratification and nutrient supply. Increased temperature, enhanced surface stratification, nutrient upwelling, stimulation of photosynthesis by elevated CO₂, changes in land runoff and nutrient availability, and altered ocean pH may produce contradictory species- or even strain-specific responses. Complex factor interactions exist, and ecophysiological experiments rarely take into account genetic strain diversity and physiological plasticity.

Predicting the impact of global climate change on harmful algal blooms is fraught with uncertainties. However, important lessons can be learned from the dinoflagellate cyst fossil record (Dale, 2001) and from the few long-term data sets available (such as the Continuous Plankton Recorder surveys; Hays, Richardson and Robinson, 2005; Figures 34 and 35). The climate on our planet has been forever changing, from scales of millions of years (glacial to interglacial periods) to short-term oscillations of tens of years (ENSO, and the North Atlantic Oscillation). Even in the past 1 000 years, the planet has gone through episodes much warmer than present (the Medieval Warm Period of 550–1300 AD) or much colder than now (the Little Ice Age 1300–1900 AD). Because of their short generation times and longevity, many phytoplankton can respond to climate change with only a very small time lag. They can spread quickly with moving water masses into climatic conditions that match the requirements of a species in terms of temperature, salinity, land runoff and turbulence.



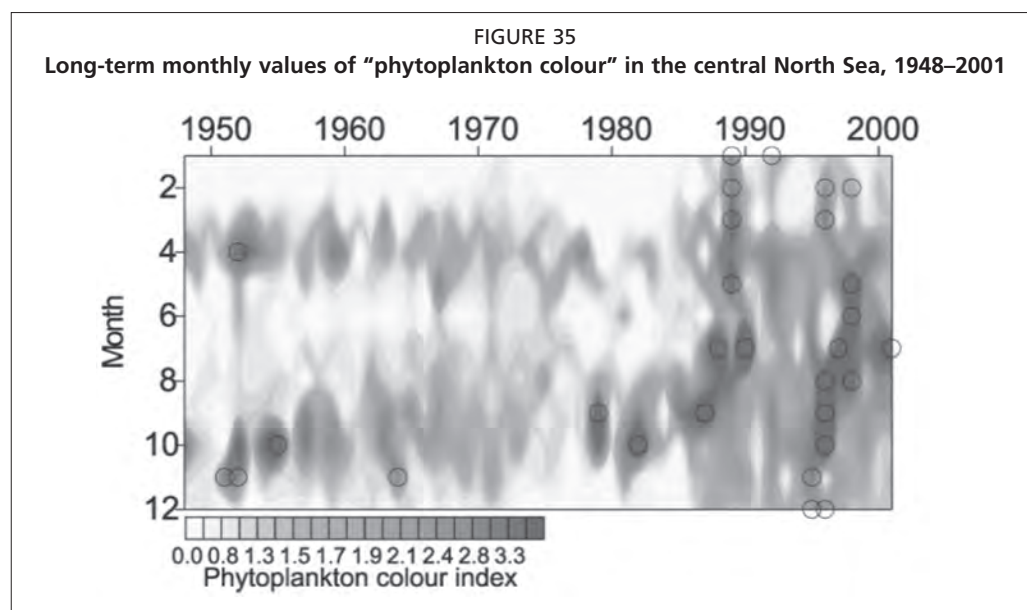
Note: Note the increase in *Prorocentrum*, *Ceratium furca* and *Dinophysis* along the Norwegian coast and increase in *Noctiluca* in the southern North Sea.

Source: Edwards *et al.* (2008).

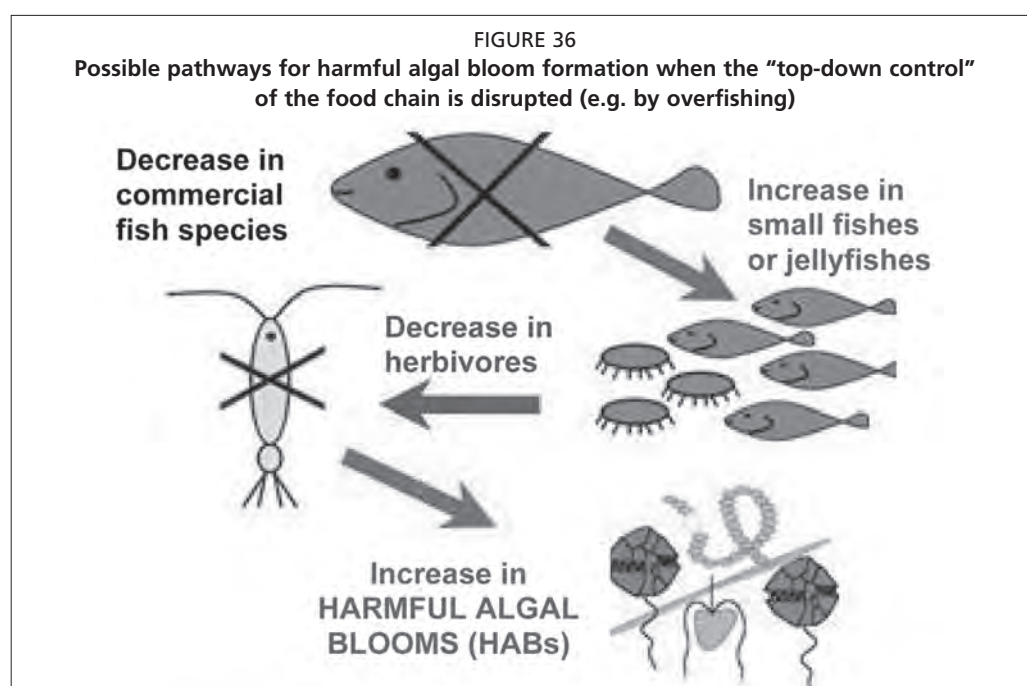
5.2.5 Impact of global warming and sea surface temperature change

Phytoplankton grow over a range of temperatures characteristic of their habitat, and growth rates are usually higher at higher temperature but considerably lower beyond an optimal temperature (Eppley, 1972). Natural populations of phytoplankton are often found at temperatures that are suboptimal for photosynthesis, and it is believed that this is designed to avoid risking abrupt declines in growth associated with the abrupt incidence of warmer temperatures (Li, 1980). Temperature effects on phytoplankton growth and composition are more important in shallow coastal waters, which experience larger temperature fluctuations than oceanic waters. Increasing sea surface temperature may shift the community composition towards species adapted to warmer temperatures, as observed in the temperate North Atlantic (Edwards and Richardson, 2004). Seasonal timing of phytoplankton blooms is now occurring up to 4–5 weeks earlier in the North Sea in relationship to regional climate warming (Figure 35). However, not all trophic levels are responding to the same extent. Where

zooplankton or fish grazers are differentially affected by ocean warming, this may have cascading impacts on the structure of marine food webs (Figure 36).



Notes: Circles denote > 2SD above the long-term monthly mean (from Edwards, 2004). Note an apparent shift towards earlier spring and autumn phytoplankton blooms.

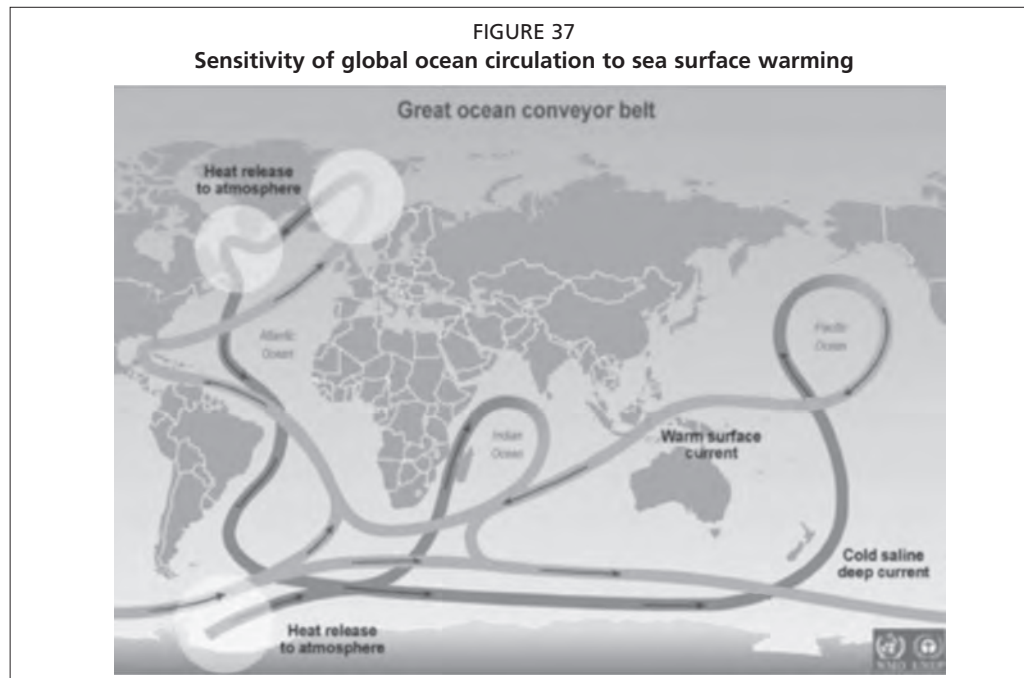


Note: Differential impacts of climate change on zooplankton or fish grazers can produce similar stimulation of harmful algal blooms.

Source: Turner and Graneli (2006).

5.2.5.1 Sea-level rise, wind and mixed-layer depth

Increasing sea surface temperature and water column stratification (shallowing of the mixed layer) can be expected to have a strong impact on phytoplankton because of the resource requirements and temperature ranges to which species are adapted. Wind determines the incidence of upwelling and downwelling, which in turn strongly affect the supply of macronutrients to the surface (recognized as drivers of *Gymnodinium catenatum* blooms off Spain [Fraga and Bakun, 1990]). Broad changes in ocean circulation such as those comprising the deep-ocean conveyor belt (Figure 37) can cause displacements to existing upwelling areas and associated algal bloom phenomena. Wind-driven currents may also transport phytoplankton away from a region, and affect the size and frequency of formation of mesoscale features such as fronts and eddies. Locally, wind intensity strongly influences the depth and intensity of vertical mixing in the surface layer, thereby affecting phytoplankton access to nutrients, light availability for algal photosynthesis and phytoplankton exposure to potentially harmful UV-B radiation. Finally, winds can influence the supply of iron to the surface ocean through aeolian transport of dust from land to sea, contributing micronutrients such as iron, which can stimulate *Karenia brevis* blooms off Florida (Walsh and Steidinger, 2001). Extreme climate events such as hurricanes are known to expand the existing distribution of cyst-producing toxic dinoflagellates (e.g. *Alexandrium tamarens* in New England, the United States of America, after a 1972 hurricane [Anderson, 1997]). Sea-level rise has the potential to increase the extent of continental-shelf areas, providing shallow, stable water columns favouring phytoplankton growth. The proliferation of coccolithophorids in the geological period the Cretaceous has been partially explained on this basis.



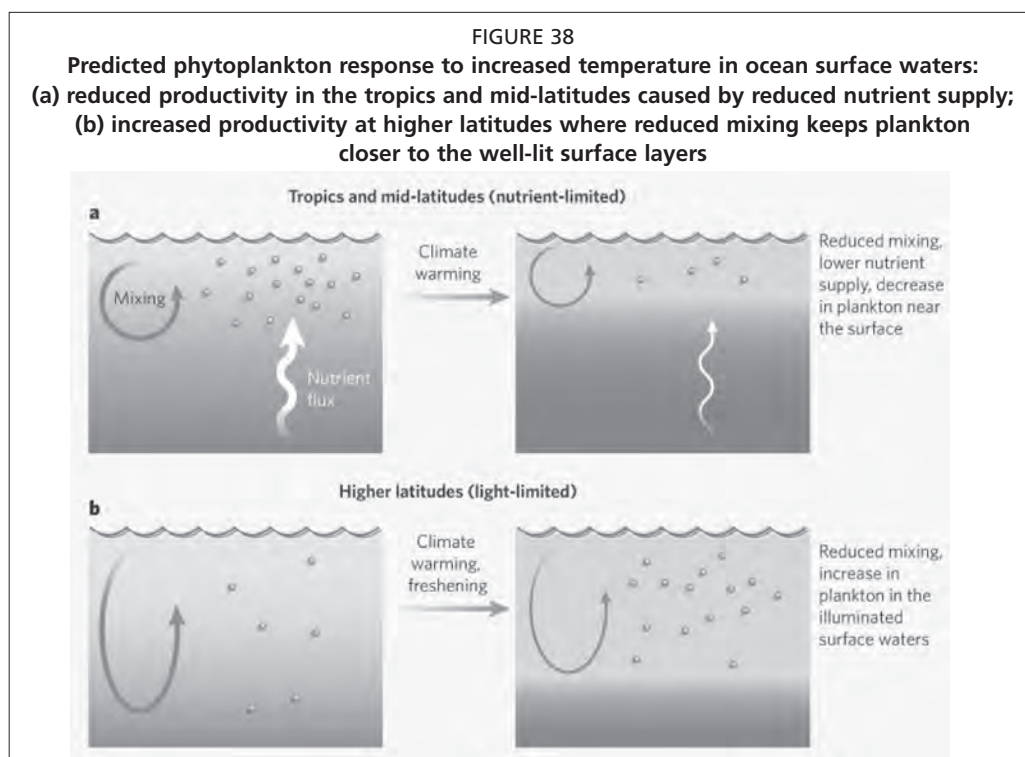
Source: Rahmstorf (2002).

5.2.5.2 Impact of heavy precipitation events and flash floods

Changes in the amount or timing of rainfall and river runoff affect the salinity of estuaries and coastal waters. Salinity is relatively constant throughout the year in most oceanic waters and in coastal areas that receive little freshwater input. Coastal phytoplankton is subject to more variation in salinity than phytoplankton in oceanic

waters. While some species grow well over a wide range of salinities, other species grow best only at salinities that are low (estuarine), intermediate (coastal) or high (oceanic species). Freshwater also modifies the stratification of the water column, thereby affecting nutrient resupply from below. While diatoms seem to be negatively affected by the decrease in nutrient concentrations associated with river discharge, dinoflagellates often benefit as this usually increases stratification and the availability of humic substances (Doblin *et al.*, 2005). PSP dinoflagellate blooms of *Gymnodinium catenatum* (in Tasmania, Australia [Hallegraeff, McCausland and Brown, 1995]) and *Alexandrium tamarense* (off Massachusetts, the United States of America [Anderson, 1997]) tend to be closely associated with land runoff events. In Hiroshima Bay, Japan, blooms of the fish-killing raphidophyte *Chattonella marina* followed typhoon-induced accretion of nutrient-rich land runoff (Kimura, Mizokami and Hashimoto, 1973).

Increased temperatures driven by climate change are predicted to lead to enhanced surface stratification, more rapid depletion of surface nutrients and a decrease in replenishment from deep nutrient-rich waters (Figure 38). This in turn will lead to a change in phytoplankton species, with smaller nanoplankton and picoplankton cells with higher surface-area:volume ratios (better able to cope with low nutrient levels) favoured over larger cells. Mixing depth affects sea surface temperature, the supply of light (from above) and nutrients (from below), and affects phytoplankton sinking losses within the surface layer. Climate models predict changes in the mixed-layer depth in response to global warming for large regions of the global ocean. In the North Pacific, decadal-scale climate and mixed-layer variability (Hayward, 1997), and, in the North Atlantic, longer-term changes in wind intensity and stratification since the 1950s have also been related to considerable changes in the phytoplankton community (Richardson and Schoeman, 2004). Similarly, in high-latitude regions with relatively deep mixing and sufficient nutrients, decreasing mixing depth has resulted in higher phytoplankton biomass because of increased light availability. In contrast, in regions with intermediate mixing depth, increased stratification has resulted in decreased phytoplankton biomass owing to reductions in nutrient supply.



Source: Doney (2006).

5.2.5.3 Ultraviolet radiation

Ultraviolet (UV) can negatively affect several physiological processes and cellular structures of phytoplankton, including photosynthesis, nutrient uptake, cell motility and orientation, algal life span, and DNA (Häder, Worrest and Kumar, 1991). Whereas shorter wavelengths generally cause greater damage per dose, inhibition of photosynthesis by ambient UV increases linearly with increasing total dose. In clear oceanic waters, UV-B radiation can reach depths of at least 30 m. Although some phytoplankton may acclimate to, compensate for, or repair damage by, UV, this involves metabolic costs, thereby reducing the energy available for cell growth and division. Raven, Finkel and Irwin (2005) suggest that UV intensity affects the size ratio in phytoplankton communities because small cells are more prone to damaging UV, and have comparatively high metabolic costs to screen it out. Many surface-dwelling red-tide species of raphidophytes and dinoflagellates possess UV-absorbing pigments, which give them a competitive advantage over species lacking such UV protection (Jeffrey *et al.*, 1999).

5.2.5.4 Ocean acidification

It is widely predicted that increasing CO₂ will lead to ocean acidification, which can potentially have an adverse impact on calcifying organisms, the most important of which in terms of biomass and carbon sequestration is the coccolithophorid *Emiliana huxleyi* (Riebesell *et al.*, 2000). Calculations based on CO₂ measurements of the surface oceans indicate that uptake by the oceans of approximately half the CO₂ produced by fossil-fuel burning has already led to a reduction in surface pH by 0.1 units. Under the current scenario of continuing global CO₂ emissions from human activities, average ocean pH is predicted to fall by 0.4 units by 2100 (Orr *et al.*, 2005). Such a pH is lower than has been experienced for millennia and, critically, this rate of change is 100 times faster than ever experienced in the known history of the planet (Royal Society, 2005). Experimental manipulations of pH in *Emiliana huxleyi* cultures have both produced reduced (Riebesell *et al.*, 2000) and enhanced calcification and growth (Iglesias-Rodriguez *et al.*, 2008). This has been partially attributed to differences in analytical procedures as well as strain-specific responses, while increasingly the potential for adaptive evolution to gradual environmental changes is now also being recognised (Lohbeck, Riebesell and Reusch, 2012).

Decreasing pH to < 8.0 has been observed to have a negative effect on nitrification in marine bacteria. Therefore, it could potentially reduce nitrate availability for plankton algae. However, the nitrogen-fixing tropical cyanobacterium *Trichodesmium* may be a beneficiary of ocean acidification (Hutchins *et al.*, 2007). Decreasing pH has also been found to increase the availability of toxic trace elements such as copper. Because the relative consumption of HCO₃⁻ and CO₂ differs between phytoplankton species, changes in their availability may affect phytoplankton on the cellular, population and community level. Most harmful algal bloom species tested thus far lack carbon-concentrating mechanisms and, hence, they may benefit from increased atmospheric CO₂, whereas diatom species such as *Skeletonema*, for which photosynthesis is already CO₂-saturated, will remain constant (Beardall and Raven, 2004).

5.2.6 Mitigation of the probable impacts on seafood safety

Our limited understanding of marine ecosystem responses to multifactorial physicochemical climate drivers, as well as our poor knowledge of the potential of marine microalgae to adapt genetically and phenotypically to the unprecedented pace of current climate change, are emphasized. Some species of harmful algae (e.g. those benefitting from increased water column stratification or increased water temperatures) may become more successful, while others may diminish in areas currently impacted (Hallegraeff, 2010). The greatest problems for human society will be caused by being

unprepared for significant range extensions of harmful algal bloom species or an increase in algal biotoxin problems in currently poorly monitored areas. While, for example, ciguatera contamination would be expected and monitored for in tropical coral-reef fish, with the apparent range extension of the causative benthic dinoflagellate into warm-temperate seagrass beds of southern Australia, other coastal fisheries could unexpectedly be at risk. Similarly, incidences of increased surface stratification in estuaries or heavy precipitation or extreme storm events are all warning signs that call for increased vigilance in monitoring seafood products for algal biotoxins, even in areas not currently known to be at risk.

6. Implementation and certification of food safety and quality systems

6.1 FOOD SAFETY OBJECTIVES (IDDYA KARUNASAGAR)

The SPS Agreement of the WTO makes provision for member countries to take measures to protect public health, and the concept of appropriate level of protection (ALOP) has been elaborated. An ALOP has been defined as the level of protection deemed appropriate by a WTO member country to establish a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory. In the food safety arena, an ALOP would be a statement of the degree of public health protection that is to be achieved by the food safety management system in the country. It could be expressed as a public health goal in terms of the disease burden (number of cases per given population over a specified period) associated with a particular hazard food combination and its consumption in a particular country. It could also be expressed in terms of achieving a reduction in the current level of food-borne illnesses. For example, the United States Healthy People 2010 programme had a goal of achieving a 50 percent reduction in illness caused by key food-borne pathogens (against a 1997 background level).¹⁰

A public health goal of reducing the current level of food-borne illness (e.g. by 50 percent) has to be translated into parameters that can be assessed by government agencies and various operators in the food chain (e.g. primary producers, processors, distributors and retailers). The food safety objective (FSO) would be a risk management tool that can be used to achieve the ALOP (CAC, 2007a). An FSO has been defined as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection (CAC, 2011). Outputs of a quantitative microbiological risk assessment would help in determining the relationship between the number of micro-organisms in foods and the risk of illness (Figure 39). For example, the FAO/WHO risk assessment for *V. vulnificus* in raw oysters estimated the risk per serving and predicted the number of cases in the susceptible population based on levels of the pathogen in oysters at consumption (Table 59). Risk assessors can also estimate reductions in illness that can be brought about by reducing the pathogen level at the point of consumption, and such an assessment could be the basis for making decisions on the FSO (Figure 39). An FSO is a target that needs to be reached, but it does not specify how this is to be achieved. This provides flexibility to food business operators to use different options to reach the target.

Adoption of good practices (GHPs, GMPs, and prerequisite programmes) and the HACCP system are tools that are used to meet FSOs. Operators in different segments of a food chain may use performance objectives at their level of the chain to assess whether the control measures adopted, for example, good practices and/or HACCP, are adequate to contribute to the FSO (Figure 39). Codex has defined a performance objective (PO) as the maximum frequency and/or concentration of a hazard in food at a specified step in the food chain before the time of consumption that provides

¹⁰ www.healthypeople.gov/document/html/volume1/10food.htm#_Toc490555746

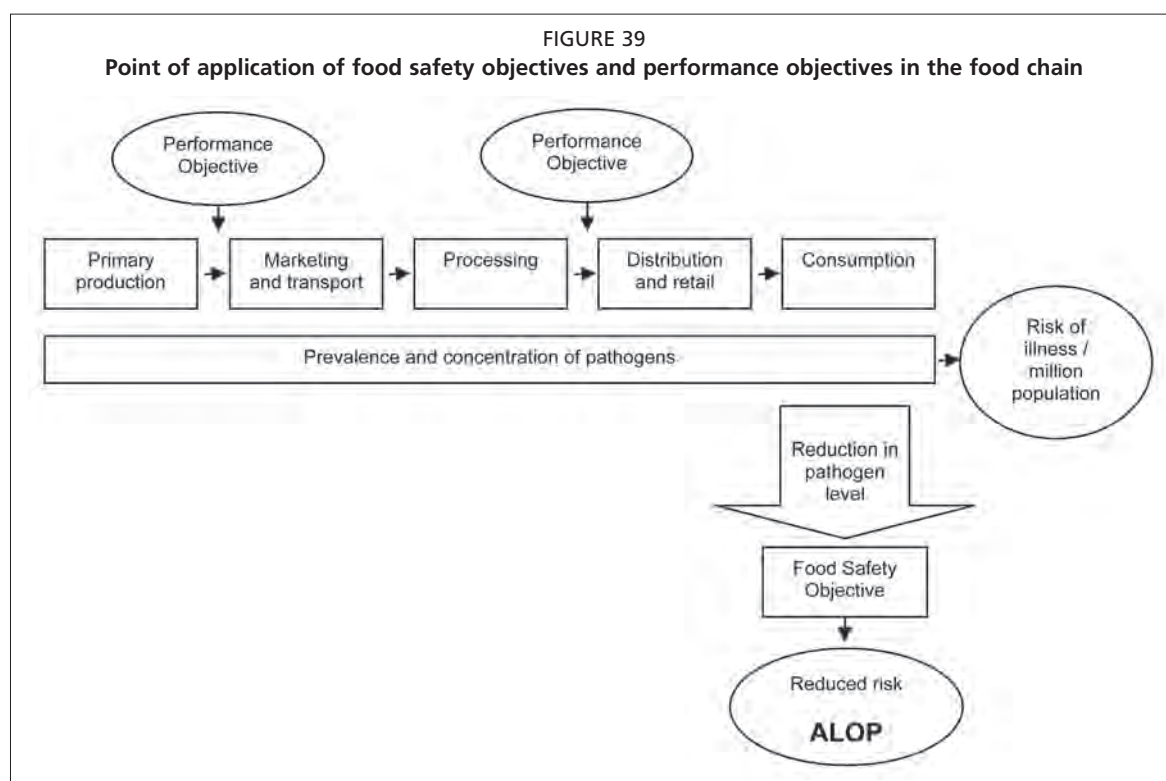
or contributes to an FSO or ALOP as applicable (CAC, 2011). Because of the link between FSOs and ALOPs, the latter to be decided by national governments, FSOs can be established only by national competent authorities. Codex may help in the establishment of FSOs through recommendations based on national and international risk assessments (CAC, 2011). Performance objectives may be set considering the changes in the level of the pathogen from the point where a PO is set and the final consumption. A PO could be stricter than an FSO if there are chances of growth of the pathogen further along in the food chain, or it may be more lenient than an FSO if the product is cooked before consumption.

TABLE 59

Summary of risk per serving and predicted number of cases in the Gulf Coast of the United States of America based on *V. vulnificus* levels in raw oysters at consumption

Month	<i>V. vulnificus</i> /g at harvest	<i>V. vulnificus</i> /g at consumption	Risk per serving for susceptible population	Predicted number of cases
Jan – Mar	40	80	1.26×10^{-6}	0.5
Apr – June	2 600	21 400	3.37×10^{-5}	11.7
Jul – Sep	5 600	57 000	4.28×10^{-5}	12.2
Oct – Dec	500	3 700	1.92×10^{-5}	8.0

Source: Extracted from FAO/WHO (2005c).



The food business operator (FBO) may use any control measure to achieve the PO. The FAO/WHO risk assessment for *V. vulnificus* in raw oysters noted that there are three validated methods to reduce this pathogen to non-detectable levels: mild-heat (50 °C) treatment, freezing with extended frozen storage and high hydrostatic pressure (FAO/WHO, 2005c). The FBO may use a performance criterion (PC) to evaluate any control option. A PC is defined by Codex as the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or FSO. An example of a PC would be a 12D reduction in proteolytic *Clostridium botulinum* in fish canning.

Compliance with a PO or FSO may sometimes be verified by microbiological testing. However, in most cases, validation of control measures, auditing good practices and HACCP, and monitoring of CCPs might be adequate to confirm that a PO and a FSO are being met. Thus, no microbiological testing is specified while setting FSOs or POs.

A microbiological criterion defines acceptability of a product or food lot based on the absence or presence or number of micro-organisms including parasites and or quantity of toxins/metabolites per units of mass, volume, area or lot (CAC, 1997). The criterion should specify the point of a food chain (e.g. at primary production, or when the product leaves the FBO) at which it is to be applied. Codex has elaborated principles for the establishment and application of microbiological criteria for foods (CAC, 1997). A microbiological criterion should be developed and applied only when there is a need as demonstrated by epidemiological evidence for public health risk due to the micro-organism or as a result of a risk assessment. Such evidence should also demonstrate that the criterion is meaningful for consumer protection. There should also be evidence that the criterion is technically attainable by applying GMPs. According to Codex (CAC, 1997), a microbiological criterion consists of: (i) a statement of the micro-organisms of concern and/or their toxins/metabolites and the reasons for the concern; (ii) the analytical methods for their detection and quantification; (iii) a plan defining the number of samples to be taken and the size of the analytical unit; (iv) the microbiological limits considered appropriate to the food at the specified point of the food chain; and (v) the number of analytical units that should conform to these limits. The criterion should also specify the food to which it applies, the point in the food chain where it applies and the actions to be taken when the criterion is not met. Some examples of Codex microbiological criteria are indicated in Table 60.

TABLE 60
Example of Codex microbiological criteria

Product	Point of application	Micro-organism	n	c	m	M	Class plan	Reference
Ready-to-eat foods in which growth of <i>L. monocytogenes</i> will not occur	End of manufacture or port of entry (for imported products), to the point of sale	<i>L. monocytogenes</i>	5	0	100 cfu/g	NA	2 class	CAC, 2007b
Ready-to-eat foods in which growth of <i>L. monocytogenes</i> can occur	End of manufacture or port of entry (for imported products), to the point of sale	<i>L. monocytogenes</i>	5	0	Absence in 25 g (< 0.04/g)	NA	2 class	CAC, 2007b
Live and raw bivalve mollusc	At the point of sale	<i>Escherichia coli</i>	5	1	230/100 g	700/100 g	3 class	CAC, 2008a
Live and raw bivalve mollusc	At the point of sale	<i>Salmonella</i>	5	0	Absence in 25 g	NA	2 class	CAC, 2008a

While deciding on microbiological criteria, consideration needs to be given to: (i) the evidence of an actual or potential hazard to health; (ii) the microbiological status of the raw material; (iii) the effect of processing on the microbiological status of the food; (iv) the likelihood and consequences of microbial contamination and/or growth during subsequent handling, storage and use; (v) the category of consumers concerned; (vi) the cost/benefit ratio associated with the application of the criterion; and (vii) the intended use of the food.

The sampling plan and microbiological methods to be used to analyse samples are to be specified in the criteria. Whenever possible, methods that have been validated for the commodity concerned and in relation to reference methods elaborated by international organizations should be used. Alternatively, methods for which reliability has been

statistically established in studies involving several laboratories should be used. Rapid methods could be used for in-plant testing and for products with a short shelf-life.

The Codex microbiological criterion for *L. monocytogenes* is an example for the criterion based on the FAO/WHO risk assessment of *Listeria monocytogenes* in RTE foods (FAO/WHO, 2004a). It estimated the risk per serving of various RTE foods, including cold-smoked fish, to susceptible populations. Based on data on the prevalence of *L. monocytogenes* in various RTE foods, the predicted distribution of the pathogen was statistically derived. The risk estimates showed that if all RTE foods had no detectable *L. monocytogenes* in 25 g (< 0.04 cfu/g), the cases of listeriosis would be 0.5 per year and that, if the levels were 100 cfu/g, there would be 5.7 cases per year. In addition, the risk characterization indicated that most cases of food-borne listeriosis occur through foods that contain more than 100 *L. monocytogenes*/g and that it is not just the numerical value of the criterion that is important but also the rate of compliance. A series of “what if” scenarios were calculated and these showed that if the percentage of “defective” servings that does not meet < 0.04 cfu/g was 0.001, there would be 119 cases, and with the 100 cfu/g criterion at the same defect rate, there would be 124 cases.

TABLE 61

Hypothetical “what if” scenario illustrating the predicted number of listeriosis cases at assumed defective rates and microbiological criteria

Assumed % of “defective” servings ¹	Predicted number of listeriosis cases ²	
	Initial standard of 0.04 cfu/g	Initial standard of 100 cfu/g
0	0.5	5.7
0.00001	1.7	6.9
0.0001	12.3	17.4
0.001	119	124
0.01	1 185	1 191
0.018	2 133	2 133
0.1	11 837	11 848
1	117 300	117 363

¹ For the purposes of this scenario, all defective servings were assumed to contain 106 cfu/g.

² For the purposes of this scenario, an r-value of 5.85×10^{-12} was employed and a standard serving size of 31.6 g was assumed. In the case of the 100 cfu/g calculations, the defective servings were assumed to be proportionally distributed.

Source: FAO/WHO (2004a).

The data in Table 61 show that if the microbiological criterion were set at 0.04 cfu/g and the defective rate achieved were 0.018 percent, there would be 2 133 cases. However, if the criterion were set at 100 cfu/g and a defective rate of 0.01 percent could be achieved, the number of cases would be 124, which would mean a 95 percent reduction in illness. Thus, it is not just the criterion that is important – the ability to achieve this and minimize the defective rate is extremely important. Considering that *L. monocytogenes* is an environmental organism, achieving absence of *L. monocytogenes* in foods is extremely difficult. Data from the United States of America and Europe show that the prevalence of *L. monocytogenes* is 12.8 percent in fish processing environments (Kornacki and Gurtler, 2007). Studies in Iceland and Canada showed that 3–5 percent RTE shrimp and lobster test positive for *L. monocytogenes* (Cormier *et al.*, 2007).

The FAO/WHO risk assessment also showed that growth of *L. monocytogenes* in RTE foods would greatly increase risk. For smoked fish, the prediction was a 1 231-fold increase in risk for a normal-risk population and a 1 366-fold increase for a high-risk population. It is in this context that the CAC agreed for different microbiological criteria for *L. monocytogenes* in RTE foods depending on whether the product would permit growth or not. As indicated in Table 60, in RTE foods that do

not permit growth, the *L. monocytogenes* levels should not exceed 100 cfu/g, and in RTE foods that permit growth, they should be < 0.04 cfu/g (absence in 25 g).

6.2 PREREQUISITE PROGRAMMES (LAHSEN ABABOUCHE)

The preceding chapters have shown that a large proportion of pathogens and spoilage bacteria can contaminate fish and seafood during handling, processing or distribution, either from handlers, equipment, the surrounding environment or other sources such as cleaning water or ice. To prevent this contamination from occurring, GHPs should be applied at all stages of harvesting, processing, storage and distribution. The requirements for hygienic practices constitute the prerequisite programmes that are essential for any food operation prior to the implementation of the HACCP system.

The basis for developing and implementing GHPs are the “Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1-1969, Revision 2003), Annex: HACCP System and Guidelines for its Application” and the “Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003, Revision 2008)”.

According to the Code of Practice for Fish and Fishery Products (CPFFP), the following aspects should be included in the prerequisite programme:

- fishing and harvesting vessel design and construction;
- facility design and construction;
- design and construction of equipment and utensils;
- hygiene control programme;
- personal hygiene and health;
- transportation;
- product tracing and recall procedures;
- training.

Additional useful information is available from the subsequent chapters of the CPFFP in the sections dealing with the specific fish and seafood commodities (fresh, frozen, salted, canned, etc.).

The provisions of these two Codex codes of practice have been used by most countries as the basis for their fish and seafood hygiene regulations, and by most fish and seafood trade associations and companies worldwide for drafting their food hygiene policy. For example, the FDA regulations (21CFR120.6) requires processors to have key sanitary conditions written into sanitation standard operating procedures (SSOPs), to monitor these conditions and practices, to correct unsanitary conditions and practices in a timely manner and to maintain sanitation control records. The SSOPs should address at least the following eight conditions and practices:

- safety of water and ice;
- condition and cleanliness of food contact surfaces;
- prevention of cross-contamination from unsanitary objects to food;
- maintenance of facilities for personal hygiene;
- protection of food, food packaging and food contact surfaces from adulteration;
- proper labelling, storage and use of toxic compounds;
- control of employee health conditions;
- exclusion of pests.

The written SSOP plan should explain the sanitation concerns, controls, in-plant procedures and monitoring requirements. This will demonstrate commitment to buyers and inspectors and also ensure that everyone from management to production workers understands the basics of sanitation. Likewise, the European Commission’s “Hygiene Package” addresses the prerequisite requirements both in “horizontal” legislation (Regulation 852/2004 on the hygiene of foodstuffs) and “vertical” or commodity-specific legislation (Regulation 853/2004 laying down specific hygiene

rules for food of animal origin, including fish and fishery products). The hygiene rules in “Section VIII: Fishery Products” of Regulation 853/2004, address the following:

- Chapter I. Requirements for vessels
- Chapter II. Requirements during and after landing
- Chapter III. Requirements for establishments, including vessels, handling fishery products
- Chapter IV. Requirements for processed fishery products
- Chapter V. Health standards for fishery products
- Chapter VI. Wrapping and packaging of fishery products
- Chapter VII. Storage of fishery products
- Chapter VIII. Transport of fishery products

The following is a description of the major components and requirements of a prerequisite programme. It is based mainly on the two international recommended Codex codes of practice mentioned above, with additional scientific and technical information provided as seen fit for a better understanding of how to develop and implement GHPs in a fish and seafood establishment.

6.2.1 Fishing and harvesting vessel design and construction

There are many different types of fishing vessels used throughout the world. These have evolved in particular regions to take account of the prevailing economics, environment and types of fish and shellfish caught or harvested. This section attempts to highlight the basic requirements for ease of cleaning and for minimizing fish damage, contamination and spoilage to which all vessels should have regard to the extent possible in order to ensure hygienic, high-quality handling of fresh fish.

The design and construction of a fishing vessel and of vessels used to harvest farmed fish and shellfish should take into consideration the following points.

6.2.1.1 For ease of cleaning and disinfection

- Vessels should be designed and constructed to minimize sharp inside corners and projections to avoid dirt traps.
- Construction should facilitate ample drainage.
- There should be a good supply of clean water or potable water at adequate pressure.

6.2.1.2 To minimize contamination

- All surfaces in handling areas should be non-toxic, smooth, impervious and in sound condition, to minimize the buildup of fish slime, blood, scales and guts and to reduce the risk of physical and microbial contamination.
- Where appropriate, adequate facilities should be provided for the handling and washing of fish and shellfish and should have an adequate supply of cold potable water or clean water for that purpose.
- Adequate facilities should be provided for washing and disinfecting equipment, where appropriate.
- The intake for clean water should be located to avoid contamination.
- All plumbing and waste lines should be capable of coping with peak demand.
- Non-potable water lines should be clearly identified and separated from potable water to avoid contamination.
- Objectionable substances, which could include bilge water, smoke, fuel oil, grease, drainage and other solid or semi-solid wastes, should not contaminate the fish and shellfish.

- Where appropriate, containers for offal and waste material should be clearly identified, suitably constructed with a fitted lid and made of impervious material.
- Separate and adequate facilities should be provided to prevent the contamination of fish and shellfish and dry materials, such as packaging, by poisonous or harmful substances such as oil or grease, dry storage of materials, packaging, offal and waste materials.
- Adequate hand washing and toilet facilities, isolated from the fish and shellfish handling areas, should be available where appropriate.
- Prevent the entry of birds, insects, or other pests, animals and vermin, where appropriate.

6.2.1.3 *To minimize damage to the fish, shellfish and other aquatic invertebrates*

- In handling areas, surfaces should have a minimum of sharp corners and projections.
- In boxing and shelving storage areas, the design should preclude excessive pressure being exerted on the fish and shellfish.
- Chutes and conveyors should be designed to prevent physical damage caused by long drops or crushing.
- The fishing gear and its usage should minimize damage and deterioration to the fish and shellfish.

6.2.1.4 *To minimize damage during harvesting of farmed and molluscan shellfish*

When farmed products and molluscan shellfish are harvested using seines or nets or other means and are transported live to facilities:

- Seines, nets and traps should be carefully selected to ensure minimum damage during harvesting.
- Harvesting areas and all equipment for harvesting, catching, sorting, grading, conveying and transporting of live products should be designed for their rapid and efficient handling without causing mechanical damage; these should be easily cleanable and free from contamination.
- Conveying equipment for live and slaughtered products should be constructed of suitable corrosion-resistant material that does not transmit toxic substances and should not cause mechanical injuries to the products.
- Where fish is transported live, care should be taken to avoid overcrowding and to minimize bruising.
- Where fish are held or transported live, care should be taken to maintain factors that affect fish health (e.g. carbon dioxide, oxygen, temperature, and nitrogenous wastes).

6.2.2 **Facility design and construction**

Early considerations in building a new seafood facility are the identification of a suitable location. A number of factors should be considered such as physical and geographical factors and the infrastructure available.

Among the physical needs for a facility location is a plot of adequate size (for present needs and future development), with easy access by road, rail or water. An adequate supply of potable water and energy must be available throughout the year at a reasonable cost. Special considerations must be given to waste disposal. The facility should have proper sanitary sewers. Seafood processing facilities usually produce significant quantities of organic matter that must be removed before wastewater is discharged into rivers or the sea. In addition, solid-waste handling needs careful planning, and suitable space – away from the plant – must be allocated or be available.

Assessment of the pollution risk from adjacent areas must also be considered. Contaminants such as smoke, dust, ash and foul odours (e.g. neighbouring fishmeal plant using poor-quality raw material) are obvious, but even bacteria may have to be considered as airborne contaminants (e.g. proximity of a poultry rearing plant upwind may be a source of *Salmonella* spp.).

The immediate physical surroundings of the facility should be landscaped and present an attractive appearance to the visitor. However, this should be done in a way that does not attract rodents and birds. Shrubbery should be at least 10 m away from buildings, and a grass-free strip covered with a layer of gravel or concrete should follow the outer wall of buildings. This allows for thorough inspection of walls and control of rodents. Ground immediately in front of doors and entrances should be paved to minimize dust. All areas around the facility should be well drained to prevent any standing water, where flies and micro-organisms could breed and develop.

The food facility should provide:

- adequate space for equipment, installations and storage of materials;
- separation of operations, where needed, to avoid cross-contamination;
- adequate lightning and ventilation;
- protection against pests.

External walls, roofs, doors and windows should be water-, insect- and rodent-proof. In addition, the facility should include a product flow-through pattern that is designed to prevent potential contamination, minimize process delays (which could result in further quality loss), and prevent cross-contamination between finished product and raw materials. Fish, shellfish and other aquatic invertebrates are highly perishable foods and should be handled carefully and chilled without undue delay. Equally important in the layout and design of food facilities is ensuring that there are no interruptions and no “dead ends” in the product flow, where semi-processed material can accumulate and remain for a long time at ambient temperature. Time and temperature conditions for products during processing are extremely important in preventing bacterial growth. This means that a steady and uninterrupted flow of all products is necessary in order to have full control of these critical factors. If any delays in product flow are necessary, the products should be kept chilled. Therefore, the facility should be designed to facilitate rapid processing and subsequent storage.

The design and construction of a facility should take into consideration the following points.

6.2.2.1 *For ease of cleaning and disinfection*

- The surfaces of walls, partitions and floors should be made of impervious, non-toxic materials.
- All surfaces with which fish, shellfish and their products might come into contact with should be of corrosion-resistant, impervious material that is light-coloured, smooth and easily cleanable.
- Walls and partitions should have a smooth surface up to a height appropriate to the operation.
- Floors should be constructed to allow adequate drainage.
- Ceilings and overhead fixtures should be constructed and finished to minimize the buildup of dirt and condensation, and the shedding of particles.
- Windows should be constructed to minimize the buildup of dirt and, where necessary, be fitted with removable and cleanable insectproof screens. Where necessary, windows should be fixed.
- Doors should have smooth, non-absorbent surfaces.
- Joints between floors and walls should be constructed for ease of cleaning (round joints).

6.2.2.2 *To minimize contamination*

- Facility layout should be designed to minimize cross-contamination and may be accomplished by physical or time separation between clean and unclean areas. “Unclean” areas are those where raw material is handled, often with a cleaning or preparation operation (e.g. washing, gutting, and skinning). A “clean” area is an area where any contaminant added to the product will carry over to the final product.
- Cool rooms must be separated from hot rooms where cooking, smoking, retorting, etc. are taking place. Dry rooms must be separated from wet rooms, and separate rooms must be provided for waste material, chemicals (cleaning and disinfection compounds, insecticides, all toxic materials), packaging materials and wood (for fish smoking).
- All surfaces in the handling areas should be non-toxic, smooth, impervious and in sound condition to minimize the buildup of fish slime, blood, scales and guts and to reduce the risk of physical contamination.
- Working surfaces that come into direct contact with fish, shellfish and their products should be in sound condition, durable and easy to maintain. They should be made of smooth, non-absorbent and non-toxic materials, and inert to fish, shellfish and their products, detergents and disinfectants under normal operating conditions.
- Adequate facilities should be provided for the handling and washing of products and should have an adequate supply of cold potable water for that purpose.
- Suitable and adequate facilities should be provided for storage and/or production of ice.
- Ceiling lights should be covered or otherwise suitably protected to prevent contamination by glass or other materials.
- Ventilation should be sufficient to remove excess steam, smoke and objectionable odours, and cross-contamination through aerosols should be avoided.
- Adequate facilities should be provided for washing and disinfecting equipment, where appropriate.
- Non-potable water lines should be clearly identified and separated from potable water to avoid contamination.
- All plumbing and waste lines should be capable of coping with peak demands.
- Accumulation of solid, semi-solid or liquid wastes should be minimized to prevent contamination.
- Where appropriate, containers for offal and waste material should be clearly identified, suitably constructed with a fitted lid and made of impervious material.
- Separate and adequate facilities should be provided to prevent the contamination by poisonous or harmful substances, dry storage of materials, packaging materials, offal and waste materials.
- Adequate hand washing and toilet facilities, isolated from the handling area, should be available.
- Prevent the entry of birds, insects, or other pests and animals.
- Water supply lines should be fitted with back-flow devices, where appropriate.
- Adequate lighting should be provided in all work areas.

6.2.3 **Design and construction of equipment and utensils**

The equipment and utensils used for the handling of fish and fishery products on a vessel or in a facility will vary greatly depending on the nature and type of operation involved. During use, they are constantly in contact with fish, shellfish and their products. The contact surfaces (of utensils, knives, tables, cutting boards, boxes

and containers, conveyer belts, gloves, aprons, etc.) must be designed and of such material as to be easily cleanable. Such surfaces should be constructed of non-toxic, non-absorbent material that is resistant to the environment, the food, and cleaning and disinfecting agents. Food contact materials that should be avoided are: wood, ferrous metals, brass and galvanized metals. Hayes (1992) quotes seven basic principles for hygienic design as agreed upon by food machinery professionals:

- All surfaces in contact with food must be inert to the food under the conditions of use and must not migrate to or be absorbed by the food.
- All surfaces in contact with food must be smooth and non-porous so that tiny particles of food, bacteria, or insect eggs are not caught in microscopic surface crevices and become difficult to dislodge, thus becoming a potential source of contamination.
- All surfaces in contact with food must be visible for inspection, or the equipment must be readily disassembled for inspection, or it must be demonstrated that routine cleaning procedures eliminate the possibility of contamination from bacteria or insects.
- All surfaces in contact with food must be readily accessible for manual cleaning, or if not readily accessible, then readily disassembled for manual cleaning, or if clean-in-place techniques are used, it must be demonstrated that the results achieved without disassembly are the equivalent of those obtained with disassembly and manual cleaning.
- All interior surfaces in contact with food must be so arranged that the equipment is self-emptying or self-draining.
- Equipment must be so designed as to protect the contents from external contamination.
- The exterior or non-product contact surfaces should be arranged to prevent harbouring of soils, bacteria or pests in and on the equipment itself as well as in its contact with other equipment, floors, walls or hanging supports.

Furthermore, in the design and construction of equipment, it is important to avoid dead areas where food can be trapped and bacterial growth take place. Moreover, dead ends (e.g. thermometer pockets, unused pipework, T-pieces) must be avoided, and any piece of equipment must be designed so that the product flow is always following the “first in, first out” principle.

Ease of cleaning of equipment involves a number of factors such as construction materials, accessibility and design. The most common design faults that cause poor ease of cleaning are (Shapton and Shapton, 1991):

- poor accessibility – equipment should be sited at least 1 m from a wall, ceiling or the nearest equipment;
- inadequately rounded corners – minimum radius should be 1 cm, but 2 cm is regarded as optimum by the American 3-A Sanitary Standards Committee (Hayes, 1992);
- sharp angles;
- dead ends – including poorly designed seals.

In summary, the condition of the equipment and utensils should be such that it minimizes the buildup of residues and prevents them from becoming a source of contamination. The design and construction of equipment and utensils should take into consideration the following points.

6.2.3.1 For ease of cleaning and disinfection

- Equipment should be durable and movable and/or capable of being disassembled to allow for maintenance, cleaning, disinfection and monitoring.
- Equipment, containers and utensils coming into contact with fish, shellfish and their products should be designed to provide for adequate drainage and

constructed to ensure that they can be adequately cleaned, disinfected and maintained to avoid contamination.

- Equipment and utensils should be designed and constructed to minimize sharp inside corners and projections and tiny crevices or gaps to avoid dirt traps.
- A suitable and adequate supply of cleaning utensils and cleaning agents, approved by the official agency having jurisdiction, should be provided.

6.2.3.2 *To minimize contamination*

- All surfaces of equipment in handling areas should be non-toxic, smooth, impervious and in sound condition, to minimize the buildup of fish slime, blood, scales and guts and to reduce the risk of physical contamination.
- Accumulation of solid, semi-solid or liquid wastes should be minimized to prevent contamination of fish.
- Adequate drainage should be provided in storage containers and equipment.
- Drainage should not be permitted to contaminate products.

6.2.3.3 *To minimize damage*

- Surfaces should have a minimum of sharp corners and projections.
- Chutes and conveyors should be designed to prevent physical damage caused by long drops or crushing.
- Storage equipment should be fit for the purpose and not lead to crushing of the product.

6.2.4 **Hygiene control programme**

The potential effects of harvesting and handling of products, on-board vessel handling or in-plant production activities on the safety and suitability of fish, shellfish and their products should be considered at all times.

In particular, this includes all points where contamination may exist and taking specific measures to ensure the production of a safe and wholesome product. The type of control and supervision needed will depend on the size of the operation and the nature of its activities. Schedules should be implemented to:

- prevent the buildup of waste and debris;
- protect the fish, shellfish and their products from contamination;
- dispose of any rejected material in a hygienic manner;
- monitor personal hygiene and health standards;
- monitor the pest control programme;
- monitor the cleaning and disinfecting programmes;
- monitor the quality and safety of water and ice supplies.

The hygiene control programme should take into consideration the following points.

6.2.4.1 *A cleaning and disinfection schedule*

A cleaning and disinfection schedule should be drawn up to ensure that all parts of the vessel, processing facility and equipment therein are cleaned appropriately and regularly. The schedule should be reassessed whenever changes occur to the vessel, processing facility and/or equipment. Part of this schedule should include a “clean as you go” policy.

A typical cleaning and disinfecting process may involve as many as seven separate steps:

- **Pre-cleaning:** Preparation of area and equipment for cleaning. Involves steps such as removal of all fish, shellfish and their products from the area, protection of sensitive components and packaging materials from water, removal by hand or squeegee of fish scraps, etc..

- **Pre-rinse:** A rinsing with water to remove remaining large pieces of loose soil.
- **Cleaning** means the removal of soil, food residues, dirt, grease or other objectionable matter.
- **Rinsing:** with potable water or clean water, as appropriate, to remove all soil and detergent residues.
- **Disinfection:** Application of chemicals, approved by the official agency having jurisdiction, and/or heat to destroy most micro-organisms on surfaces.
- **Post-rinse:** As appropriate, a final rinse with potable water or clean water to remove all disinfectant residues.
- **Storage:** Cleaned and disinfected equipment, container and utensils should be stored in a fashion that would prevent its contamination.
- **Checking the efficiency of cleaning:** The efficiency of the cleaning should be controlled as appropriate.

Most detergents or cleaning agents work faster and more effectively at higher temperatures, so it can be profitable to clean at a high temperature. Cleaning is often carried out at 60–80 °C in areas where it pays, in terms of energy, to use such high temperatures.

The ideal detergent would be characterized by the following properties:

- It possesses sufficient chemical power to dissolve the material to be removed.
- It has a surface tension low enough to penetrate into cracks and crevices; it should be able to disperse the loosened debris and hold it in suspension.
- If used with hard water, it should possess water-softening and calcium-salt-dissolving properties to prevent precipitation and buildup of scale on surfaces.
- It rinses freely from the surfaces, leaving them clean and free from residues, which could harm the products and affect sterilization negatively.
- It does not cause corrosion or other deterioration of surfaces. It is recommended always to check by consulting the supplier of machines, etc..
- It is not hazardous for the operator.
- It is compatible with the cleaning procedure being used, whether manual or mechanical.
- If solid, it should be easily soluble in water and its concentration easily checked.
- It complies with legal requirements concerning safety and health as well as biodegradability.
- It is reasonably economical to use.

A detergent with all these characteristics does not exist. So one must, for each individual cleaning operation, select a compromise by choosing a usable cleaning agent and water treatment additives so that the combined detergent has the properties that are most important for the procedure concerned.

All cleaning methods, including foams and soaks, require sufficient contact time to fully loosen and suspend soils. A moderately alkaline detergent, which is normally used in plants processing high-protein foods such as fish, will typically require 10–15 min to fully loosen most processing soils.

Disinfection can be effected by physical treatments such as heat, UV irradiation, or by means of chemical compounds.

The use of heat in the form of steam or hot water is a very safe and widely used method of disinfection. The most commonly used chemicals for disinfection are shown in Table 62.

Chlorine is one of the most effective and widely used disinfectants. It is available in several forms, for example, sodium hypochlorite solutions, chloramines and other chlorine-containing organic compounds. Gaseous chlorine and chlorine dioxide are also used. Chlorinated disinfectants at a concentration of 200 ppm free chlorine are

very active and have a cleaning effect. The disinfectant effect is considerably decreased when organic residues are present. The compounds dissolved in water will produce hypochlorous acid, which is the active disinfecting agent, acting by oxidation. In solution it is very unstable, particularly in acid solution, where toxic chlorine gas will be liberated. Moreover, solutions are more corrosive at low pH.

As the germicidal activity is considerably better in acid than in alkaline solution, the working pH should be chosen as a compromise between efficiency and stability. Organic chlorinated disinfectants are generally more stable but require longer contact times. When used in the proper range of values (200 ppm free chlorine), chlorinated disinfectants in solutions at ambient temperatures are non-corrosive to high-quality stainless steel, but they are corrosive to other less-resistant materials.

TABLE 62
Types of disinfectants

Disinfectant	Forms/ description	Advantages	Disadvantages
Chlorine	Hypochlorites, chlorine gas, organic chlorine, e.g. chloramines	Kills most types of micro-organisms Less affected by hard water than some Does not form films Effective at low temperatures Relatively inexpensive Concentration easily determined by test strips	May corrode metals and weaken rubber Irritating to skin, eyes and throat Unstable, dissipates quickly Liquid chlorine loses strength in storage pH-sensitive
Iodophors	Iodine dissolved in surfactant and acid	Kills most types of micro-organisms Less affected by organic matter than some Less pH-sensitive than chlorine Concentration determined by test strips Solution colour indicates active sanitizer	May stain plastics and porous materials Inactivated above 50 °C Reduced effectiveness at alkaline pH More expensive than hypochlorites May be unsuitable for CIP* due to foaming
Quaternary ammonium compounds	Benzalkonium chloride and related compounds, sometimes called quats or QACs	Non-corrosive Less affected by organic matter than some Residual antimicrobial activity if not rinsed Can be applied as foam for visual control Effective against <i>Listeria monocytogenes</i> Effective for odour control Concentration determined by test strips	Inactivated by most detergents May be ineffective against certain organisms May be inactivated by hard water Effectiveness varies with formulation Not as effective at low temperature as some May be unsuitable for CIP due to foaming
Acid-anionic	Combination of certain surfactants and acids	Sanitize and acid rinse in one step Very stable Less affected by organic matter than some Can be applied at high temperature Not affected by hard water	Effectiveness varies with micro-organism More expensive than some pH-sensitive (use below pH 3.0) Corrode some metals May be unsuitable for CIP due to foaming
Peroxy compounds	Acetic acid and hydrogen peroxide combined to form peroxyacetic acid	Best against bacteria in biofilms Kills most types of micro-organisms Relatively stable in use Effective at low temperatures Meets most discharge requirements Low foaming; suitable for CIP	More expensive than some Inactivated by some metals/organics May corrode some metals Not as effective as some against yeasts and moulds

TABLE 62 (continued)

Disinfectant	Forms/ description	Advantages	Disadvantages
Carboxylic acid	Fatty acids combined with other acids; sometimes called fatty acid sanitizers	Kills most types of bacteria Sanitize and acid rinse in one step Low foaming, suitable for CIP Stable in presence of organic matter Less affected by hard water than some	Inactivated by some detergents pH-sensitive (use below pH 3.5) Less effective than chlorine at low temperature May damage non-stainless-steel materials Less effective against yeasts and moulds than some
Chlorine dioxide	A gas formed on-site and dissolved in solution or by acidification of chlorite and chlorate salts	Kills most type of micro-organisms Stronger oxidizer (sanitizer) than chlorine Less affected by organic matter than some Less corrosive than chlorine Less pH-sensitive than some	Unstable and cannot be stored Potentially explosive and toxic Relatively high initial equipment cost
Ozone	A gas formed on-site and dissolved in solution	Kills most type of micro-organisms Stronger oxidizer (sanitizer) than chlorine and chlorine dioxide	Unstable and cannot be stored May corrode metals and weaken rubber Potentially toxic Inactivated by organic matter (similar to chlorine)
Hot water / heated solutions	Water at 77–88 °C	Kills most types of micro-organisms Penetrates irregular surfaces Suitable for CIP Relatively inexpensive	May form films or scale on equipment Burn hazard Contact time-sensitive

* CIP = cleaning in place.

Source: After Anon. (2000b).

Iodophors contain iodine, bound to a carrier, usually a non-ionic compound, from which the iodine is released for sterilization. Normally, the pH is brought down to 2–4 by means of phosphoric acid. Iodine has its maximum effect in this pH range.

Iodophors are active disinfectants with a broad antimicrobial spectrum such as chlorine. They are inactivated by organic material. Concentrations corresponding to approximately 25 ppm free iodine will be effective.

Commercial formulations are often acidic, making them able to dissolve scales. They can be corrosive depending on the formulation and they should not be used above 45 °C as free iodine may be liberated. If residues of product and caustic cleaning agents are left in dead ends and similar places, this may, in combination with iodophores, cause very unpleasant “phenolic” off-flavours.

Hydrogen peroxide and **peracetic acid** are effective disinfectants acting by oxidation and with a broad antimicrobial spectrum. Diluted solutions may be used alone or in combination for disinfection of clean surfaces. They lose their activity more readily than other disinfectants in the presence of organic substances and they rapidly lose their activity with time. They should be used in concentrations of 200–300 ppm.

Quaternary ammonium compounds are cationic surfactants. They are effective fungicides and bactericides but are often less effective against Gram-negative bacteria. To avoid development of resistant strains of micro-organisms, these compounds should only be used by alternating with the use of other types of disinfectants.

Because of their low surface tension, they have good penetrating properties and, for the same reason, they can be difficult to rinse off. If quaternary ammonium compounds come into contact with anion-active detergents, they will precipitate and become inactivated. Therefore, mixing or successive use of these two types of chemicals must

be avoided. They can be used in concentrations of 200 ppm on food contact surfaces. Table 63 summarizes the concentrations of commonly used disinfectants.

TABLE 63
Disinfectant concentrations commonly used in food plants

Disinfectant	Food contact surface	Non-food contact surfaces	Plant water
Chlorine	100–200 ppm ¹	400 ppm	3–10 ppm
Iodine	25 ppm ¹	25 ppm	
Quaternary ammonium compounds	200 ppm ¹	400–800 ppm	
Chlorine dioxide	100–200 ppm ^{1,2}	100–200 ppm ²	1–3 ppm ²
Peroxyacetic acid	200–315 ppm ¹	200–315 ppm	

¹ The higher end of the listed range indicates the maximum concentration permitted without a required rinse (surfaces must drain).

² Includes mix of oxychloro compounds.

Source: Anon. (2000b).

6.2.4.2 Designation of personnel for cleaning

Handlers or cleaning personnel as appropriate should be well trained in the use of special cleaning tools and chemicals, and in the methods of dismantling equipment for cleaning. They should be knowledgeable about the significance of contamination and the hazards involved.

In each processing plant or vessel, a trained individual should be designated to be responsible for the sanitation of the processing facility or vessel and the equipment therein.

6.2.5 Maintenance of premises, equipment and utensils

- Buildings, materials, utensils and all equipment in the establishment – including drainage systems – should be maintained in a good state and order.
- Equipment, utensils and other physical facilities of the plant or vessel should be kept clean and in good repair.
- Procedures for the maintenance, repair, adjustment and calibration, as appropriate, of apparatus should be established. These procedures should specify for each piece of equipment, the methods used, the persons in charge of their application, and their frequency.

6.2.6 Pest control systems

- Good hygiene practices should be employed to avoid creating an environment conducive to pests.
- Pest control programmes could include preventing access, eliminating harbourage and infestations, and establishing monitoring detection and eradication systems.
- Physical, chemical and biological agents should be properly applied by appropriately qualified personnel.

6.2.7 Supply of water, ice and steam

Water:

- An ample supply of cold and hot potable water and/or clean water under adequate pressure should be provided where appropriate.
- Potable water should be used wherever necessary to avoid contamination.

Ice:

- Ice should be manufactured using potable water or clean water.
- Ice should be protected from contamination.

Steam:

- For operations that require steam, an adequate supply of water at sufficient pressure should be maintained.
- Steam used in direct contact with fish or shellfish or food contact surfaces should not constitute a threat to the safety or suitability of the food.

6.2.8 Waste management

- Offal and other waste materials should be removed from the premises of a processing facility or vessel on a regular basis.
- Facilities for the containment of offal and waste material should be properly maintained.
- Vessel waste discharge should not contaminate vessel water-intake systems or incoming product.

6.2.9 Personal hygiene and health

- Personal hygiene and facilities should be such to ensure that an appropriate degree of personal hygiene can be maintained to avoid contamination.

6.2.9.1 Facilities and equipment

Facilities and equipment should include:

- Adequate means for hygienically washing and drying hands.
- Adequate toilet and changing facilities for personnel should be suitably located and designated.

6.2.9.2 Personnel hygiene

- No person who is known to be suffering from, or who is a carrier of, any communicable disease or has an infected wound or open lesion should be engaged in the preparation, handling or transportation of fish.
- Where necessary, adequate and appropriate protective clothing, head covering and footwear should be worn.
- All persons working in a facility should maintain a high degree of personal cleanliness and should take all necessary precautions to prevent contamination of products or facilities.
- Hand-washing should be carried out by all personnel working in a processing area:
 - at the start of fish or shellfish handling activities and upon re-entering a processing area;
 - immediately after using the toilet.
- The following should not be permitted in handling and processing areas: smoking, spitting, chewing or eating, sneezing or coughing over unprotected food.
- The adornment of personal effects such as jewellery, watches, pins or other items that, if dislodged, may pose a threat to the safety and suitability of the products should not be allowed.

6.2.10 Transportation

Vehicles should be designed and constructed:

- Such that walls, floors and ceilings, where appropriate, are made of a suitable corrosion-resistant material with smooth non-absorbent surfaces. Floors should be adequately drained.
- Where appropriate, with chilling equipment to maintain chilled fish or shellfish during transport to a temperature as close as possible to 0 °C or, for frozen fish, shellfish and their products, to maintain a temperature of -18 °C or colder

- (except for brine frozen fish intended for canning, which may be transported at -9°C or colder).
- Live fish and shellfish are to be transported at temperatures that the species can tolerate.
 - To provide the fish or shellfish with protection against contamination, exposure to extreme temperatures and the drying effects of the sun or wind.
 - To permit the free flow of chilled air around the load when fitted with mechanical refrigeration means.

6.2.11 Product tracing and recall procedures

Experience has demonstrated that a system for recall of product is a necessary component of a prerequisite programme. Product tracing, which includes lot identification, is essential to an effective recall procedure. Therefore:

- Managers should ensure effective procedures are in place to effect the complete product tracing and rapid recall of any lot of fishery product from the market.
- Appropriate records of processing, production and distribution should be kept and retained for a period that exceeds the shelf-life of the product.
- Each container of fish, shellfish and their products intended for the final consumer or for further processing should be clearly marked to ensure the identification of the producer and of the lot.
- Where there is a health hazard, products produced under similar conditions, and likely to present a similar hazard to public health, may be withdrawn. The need for public warnings should be considered.
- Recalled products should be held under supervision until they are destroyed, used for purposes other than human consumption, or reprocessed in a manner to ensure their safety.

6.2.12 Training

Fish or shellfish hygiene training is of fundamental importance. All personnel should be aware of their role and responsibility in protecting fish or shellfish from contamination and deterioration. Handlers should have the necessary knowledge and skill to enable them to handle fish or shellfish hygienically. Those who handle strong cleaning chemicals or other potentially hazardous chemicals should be instructed in safe handling techniques.

Each fish and shellfish facility should ensure that individuals have received adequate and appropriate training in the design and proper application of an HACCP system and process control. Training of personnel in the use of HACCP is fundamental to the successful implementation and delivery of the programme in fish or shellfish processing establishments. The practical application of such systems will be enhanced when the individual responsible for HACCP has successfully completed a course. Managers should also arrange for adequate and periodic training of relevant employees in the facility so that they understand the principles involved in HACCP.

6.3 PRINCIPLES AND APPLICATION OF THE HAZARD ANALYSIS CRITICAL CONTROL POINT SYSTEM (LAHSEN ABABOUCHE)

6.3.1 Introduction

The Hazard Analysis Critical Control Point (HACCP) system identifies, evaluates and controls hazards that are significant for food safety (CAC, 1969, 2003). It is a science-based and systematic tool that assesses hazards and establishes control systems that focus on prevention rather than rely mainly on end-product testing. It not only has the advantage of enhancing the safety of the product but, because of the means of documentation and control, it provides a way for demonstrating competence to customers and compliance with legislative requirements to the food control authorities.

Credit for the development of HACCP is traditionally given to the 1971 United States Food Protection Conference, with the first industry application by the Pillsbury Company in the 1960s for astronaut feeding during the inception of the NASA manned space programme. However, the basic concepts of HACCP are found in the Hazard Opportunity Studies, which have been used by the chemical and engineering industries for hazard controls since the mid-1930s. The following are important dates for the development of HACCP:

- 1971: The HACCP concept presented at the United States National Conference on Food Protection.
- 1973: Comprehensive treatise on HACCP published by the Pillsbury Co. HACCP – with only three principles.
- 1980: Joint WHO/ICMSF report on HACCP.
- 1983: WHO Europe recommends HACCP.
- 1985: National Academy of Sciences (the United States of America) recommends HACCP.
- 1988: Book on HACCP published by the ICMSF.
- 1989: The National Advisory Committee on Microbiological Criteria for Foods (NACMCF), the United States of America, approves the first major document on HACCP.
- 1992: The NACMCF issues a revised document on HACCP, whereby HACCP has seven principles.
- 1993: Codex issues the first HACCP Guidelines, which are adopted by the FAO/WHO Codex Alimentarius Commission (CAC).
- 1997: Based on a number of FAO/WHO consultations, Codex issues a revised document. The NACMCF issues its third, revised document. The two revised documents from Codex and the NACMCF are very similar.
- 2003: Based on the work of the CCFFP, the CAC adopts the first edition of the Code of Practice for Fish and Fishery Products (CPFFP), which includes requirements for prerequisites and HACCP development for 12 fish and seafood commodities, including aquaculture. Latest revision: 2008.

Likewise, the integration of HACCP into the official regulations of many countries, including major fish and seafood importers, took place as follows:

- 1991: The European Commission adopts Council Directive no. 91/493/EEC (EC, 1991), which places the responsibility of product safety and quality on the industry and introduces the concept of “own checks” and CCPs during processing.
- 1992: Canada adopts the Quality Management Program based on the HACCP principles.
- 1993: European Union Council Directive no. 93/43/EEC (EC, 1993) on the hygiene of foodstuffs is adopted, mandating only the five first principles for HACCP.
- 1994: Commission Decision 94/356/EEC (EC, 1994) detailing the rules for the application of the HACCP system is adopted.
- 1995: United States FDA (FDA, 1995) issues the Code of Federal Regulations on safe and sanitary processing and importing of fish and fishery products.
- 1996: United States Department of Agriculture, Food Safety and Inspection Service adopts the final rule on the HACCP system (USDA, 1996).
- 1992–99: With financial support from Danida, FAO implements a global programme for training government and industry staff on the application of HACCP in fisheries and aquaculture.
- 1997: Canadian Food Inspection Agency Act, which establishes the Canadian Food Inspection Agency.
- 2003: Food Safety Basic Law comes into force in Japan.

- 2004: European Union Food Hygiene Regulations come into force – including: Regulation EC/852/2004, which lays down the general hygiene requirements for all food business operators; Regulation EC/853/2004, which lays down additional specific requirements for food businesses dealing with foods of animal origin, including live bivalve molluscs and fishery products; Regulation EC/854/2004, which lays down the official controls for foods of animal origin (EC, 2004a, 2004b, 2004c). The basis for the Regulations is set down by the General Food Law Regulation EC/178/2002 (EC, 2002a).
- 2011: United States Food Safety Modernisation Act – stronger enforcement of food safety measures to better protect public health. Includes new regulatory tools and enforcement authorities.

From the food industry perspective, following the introduction of HACCP in 1971, the food canning industry in the United States of America and the FDA quickly adopted the preventive controls and the documentation aspects of HACCP. Other segments of the food industry voluntarily and gradually introduced HACCP, or elements of HACCP, into their food safety and quality assurance programmes. Starting in the mid-1980s, as HACCP became a major focus of regulatory agencies and industry in the United States of America, Europe, Canada, New Zealand, Australia and other countries, it was clearly established that HACCP had to be an industry-driven programme, with regulatory and control agencies being in charge of certifying the food facilities and conducting on-site verification of proper HACCP implementation.

Since then, HACCP has been in a constant state of evolution. Implementation by the food industry has been slow and at times painful – and it is a process that is still in progress. Application guidelines, prerequisite programmes, decision trees and training programmes have been developed and implemented. Coalition of industries, such as the United States Seafood HACCP Alliance or the Seafood Services Australia (SSA), have been formed to train and certify HACCP trainers, develop hazard analysis and generic HACCP plans.

Currently, most national food control agencies and international institutions have adopted regulations, guidelines, codes and procedures for the development and implementation of HACCP plans by industry. As a consequence of HACCP becoming the food safety regulatory system of choice, policy issues have been shaping its evolution, sometimes more than science. For the future, it is important to ensure that food safety policy frameworks maintain the science basis at the heart of HACCP development to embrace future technological developments and the food safety challenges they will bring.

Many books and articles on the principles and the application of HACCP have been published since the advent of HACCP. One guide specific to fish and fishery products is the “Fish and Fishery Products Hazards and Controls Guidance” from the United States Food and Drug Administration.¹¹

The present chapter is intended as a general introduction to HACCP, giving sufficient information to reader to understand the system and to enable them to apply or assess the system in practical fish and seafood safety assurance programmes. It reviews the basic definitions and principles of HACCP and describes how these principles can be applied in the fish and aquaculture industry.

The HACCP system can be used to deal with both safety and quality issues, although some regulatory agencies, such as the FDA, have confined it to safety aspects. Experts in food microbiology argue that, given that many control measures (e.g. hygiene, refrigeration, use of ice, and thermal treatment) actually prevent the

¹¹ Fish and Fishery Products Hazards and Controls Guidance. Fourth Edition. November 2011. U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition.

growth of micro-organisms of concern to both safety and quality, it is advisable to use HACCP to address both aspects. The additional burden is related to the expansion of record-keeping and documentation to address both safety and quality, and, consequently the additional time and personnel needed to verify and audit these records by the food control authorities. This chapter addresses only the safety aspects of HACCP for illustrative purposes. The CPFFP (described in Chapter 5; and CAC, 2003) addresses both safety CCPs and DAPs.

6.3.2 Basic principles of HACCP

The CAC has adopted the basic texts on food hygiene, including HACCP, and the guidelines for the application of HACCP were revised in 2003 (CAC/RCP 1-1969, Revision 2003). The following definitions and basic principles are based on the Codex-adopted documents.

6.3.2.1 Definitions

Control (verb): To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.

Control (noun): The state wherein correct procedures are being followed and criteria are being met.

Control measure: Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action: Any action to be taken when the results of monitoring at the CCP indicate a loss of control.

Critical control point (CCP): A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit: A criterion that separates acceptability from unacceptability.

Deviation: Failure to meet a critical limit.

Flow diagram: A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

HACCP: A system that identifies, evaluates, and controls hazards that are significant for food safety.

HACCP plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.

Hazard: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which hazards are significant for food safety and therefore should be addressed in the HACCP plan.

Monitor: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

Step: A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.

Validation: Obtaining evidence that the elements of the HACCP plan are effective.

Verification: The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan.

6.3.2.2 The HACCP system

The HACCP system can be applied from production to consumption. It consists of the following seven principles:

Principle 1: Conduct a hazard analysis	Identify the potential hazard(s) associated with each stage of production; assess the likelihood of occurrence of the hazard and identify the measures for their control.
Principle 2: Determine critical control points (CCPs)	Determine the points, procedures or operational steps that can be controlled to eliminate the hazard(s) or minimize its (their) likelihood of occurrence.
Principle 3: Establish critical limit(s)	Establish critical limit(s), which must be met to ensure that the CCP is under control.
Principle 4: Establish a system to monitor control of the CCP	Establish a system to monitor control of the CCP by scheduled testing or observations.
Principle 5: Establish corrective action(s)	Establish the corrective action(s) that must be taken when monitoring indicates that a particular CCP is not under control.
Principle 6: Establish procedures for verification	Establish procedures for verification including supplementary tests and procedures to confirm that the HACCP system is working effectively.
Principle 7: Establish records and record-keeping	Establish documentation concerning all procedures and records appropriate to these principles and their application.

6.3.3 Development of HACCP plans

Prior to the application of HACCP to a fish or seafood establishment, that establishment should be operating proper prerequisite programmes according to the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1-1969, Revision 2003) Annex: HACCP System and Guidelines for its Application and according to the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003, Revision 2008). These prerequisite programmes and the modalities of their application in the fish and aquaculture industry have been described in detail above.

Management awareness and commitment are necessary for the implementation of an effective HACCP system. The effectiveness will also rely upon management and employees having the appropriate HACCP knowledge and skills. Therefore, ongoing training is necessary for all levels of employees and managers, as appropriate.

If the necessary expertise is not available on-site for the development and implementation of an effective HACCP plan, expert advice should be obtained from other sources, such as trade and industry associations, independent experts and regulatory authorities. HACCP literature and fish and seafood HACCP guides can be valuable and they provide a useful tool for businesses in designing and implementing the HACCP plan.

The application of HACCP principles consists of the following tasks as identified in the logic sequence for the application of HACCP (CAC, 2003).

1. Assemble the HACCP team.
2. Describe product.
3. Identify intended use.
4. Construct flow diagram.
5. Confirm flow diagram.
6. Conduct hazard analysis.
7. Determine CCPs (decision tree).
8. Establish critical limits for each CCP.
9. Establish a monitoring system for each CCP.
10. Establish corrective action.
11. Establish verification procedures.
12. Establish documentation and record-keeping.

An HACCP plan is a final document that describes how a fish or seafood operation will manage the identified CCPs for each product under its particular environment and

working conditions. The following are the details on how to apply the above sequence for the preparation of a specific HACCP plan.

6.3.3.1 *Assemble an HACCP team*

A qualified HACCP team should be put together with the view to develop the HACCP plan. It should have expertise in food safety and quality, food technology and quality assurance. If the necessary knowledge and skills are not available at the seafood operation, the team can be assisted by local public health officers, independent experts, and fish inspection or fisheries extension officers. Technical advice provided to small operators by companies that buy raw material for further handling, processing or distribution is a valuable alternative, especially in the case of small-scale aquaculture or artisanal fishing.

The HACCP team should have access to all relevant and necessary information. The previous chapters of this publication are a good source of information for the HACCP team to identify the hazards and the control measures. Additional information can be found from various sources, the most relevant to the fish and aquaculture industry are referenced in this document.

For example, A HACCP team of a hypothetical seafood operation can be formed by:

- The safety and quality supervisor, with a degree/training in food science/food safety, good experience in the production/processing operations and a special training in HACCP application in the fish industry.
- The technical supervisor, with a degree/training in food technology, experience in seafood industry and a special training in HACCP application in the fish industry.
- The equipment maintenance supervisor.
- Key personnel such as the retort or double-seam supervisor in a cannery.
- As appropriate, an advisor on fish and seafood safety and quality assurance.

6.3.3.2 *Describe the product*

A full description of the product should be drawn up, including relevant safety information such as:

- harvesting area and technique;
- raw materials and ingredients used including commercial and Latin name of the fish;
- factors that influence safety such as composition, physical/chemical parameters, such as water activity (a_w), pH, salt content;
- processing such as heating, freezing, brining or smoking;
- packaging type;
- storage conditions and methods of distribution;
- shelf-life under specified condition should also be recorded.

An example of product description for depurated oysters can be as follows:

“Live oysters (*Crassostrea gigas*) harvested from (locality), depurated for at least 44 hours, using UV disinfected water. The depurated oysters are packed in mesh nets and sold live to retailers and to restaurants.

6.3.3.3 *Identify intended use*

The intended use should be based on the expected uses by the end user or consumer. The use and preparation before use greatly influence the safety of the product. Certain products may carry harmful organisms as part of the natural flora. If the processing does not include a killing step, the only possibility to render the product safe is adequate heat treatment (e.g. cooking) during preparation. It is important to identify whether the product is to be used in a way that increases the risk of harm to the consumer, or whether the product is particularly used by consumers who are especially susceptible to a hazard. In specific cases, e.g. institutional feeding, vulnerable groups of the population, such as elderly and infants, must be considered.

For example, a description of the intended use can read as follows:

“The food, live carp, is harvested from earthen ponds, packed in ice in plastic boxes, and distributed to wholesale or retail markets or to fish processing plants. It is consumed after cooking or frying.”

or

“The product, canned tuna in olive oil, is destined for export mainly to Europe and the United States of America. It is generally consumed without any cooking, as an appetizer, in a sandwich or after mixing with other food or salads. It is consumed by the public at large, with no specific age restriction.

6.3.3.4 *Construct flow diagram*

A flow diagram should be constructed by the HACCP team to provide a clear and simple description of all steps involved in the operation. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specific operation. Receiving and storage steps for raw materials and ingredients should be included. Time and temperature conditions during processing should be mentioned whenever there is a holding step, e.g. in holding vats, buffer tanks or other areas, where there could be a potential delay or temperature abuse.

6.3.3.5 *On-site verification of flow diagram*

The HACCP team should confirm on-site the production operations against the flow diagram and amend it with information, such as correct durations, temperatures, and salt concentration, where appropriate. The site should be inspected during all hours (including night shifts and weekends) of operation to check for correctness and ensure that nothing crucial has been overlooked.

6.3.3.6 *List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards (see Principle 1)*

A hazard is defined as a biological, chemical or physical agent in, or condition of, food (e.g. temperature abuse, insufficient thermal process), with the potential to cause an adverse health effect and harm.

The HACCP team should list all hazards that may reasonably be expected to occur during production, processing, transportation and distribution until the point of fish consumption.

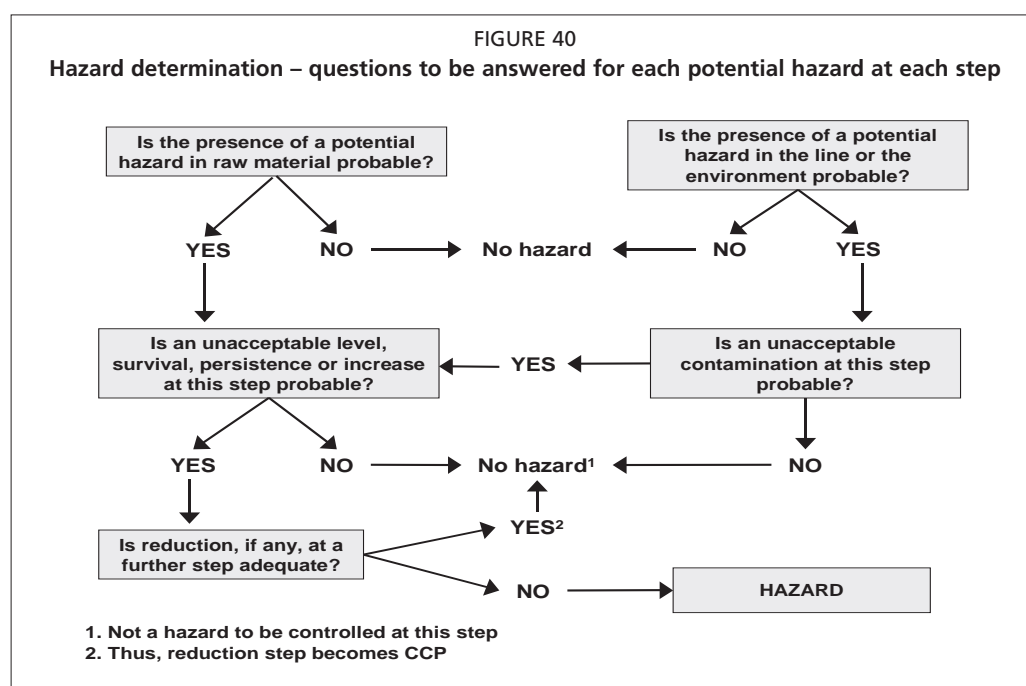
Hazard analysis is the first HACCP principle and the science-based component of HACCP. An inaccurate hazard analysis would inevitably lead to the development of an inadequate HACCP plan.

The HACCP team should identify which hazards are of such a nature that their elimination or reduction to acceptable levels is essential for the production of a safe product.

Examples of questions to be considered, when conducting a hazard analysis are as follows:¹²

- Raw materials and ingredients – do they contain any hazardous agents?
- Intrinsic factors – will the seafood permit survival, multiplication of pathogens or toxin formation?
- Processing conditions – are contaminants or pathogens reduced or destroyed, are there any possibilities for recontamination?
- Packaging – does the packaging affect the microbial population? (e.g. vacuum packaging favours anaerobes)
- Preparation and intended use – will the food be heated or cooked before consumption?
- Intended consumer – is the product destined for the general public or for consumption by a population with higher susceptibility to illness such as infants, elderly people or patients?

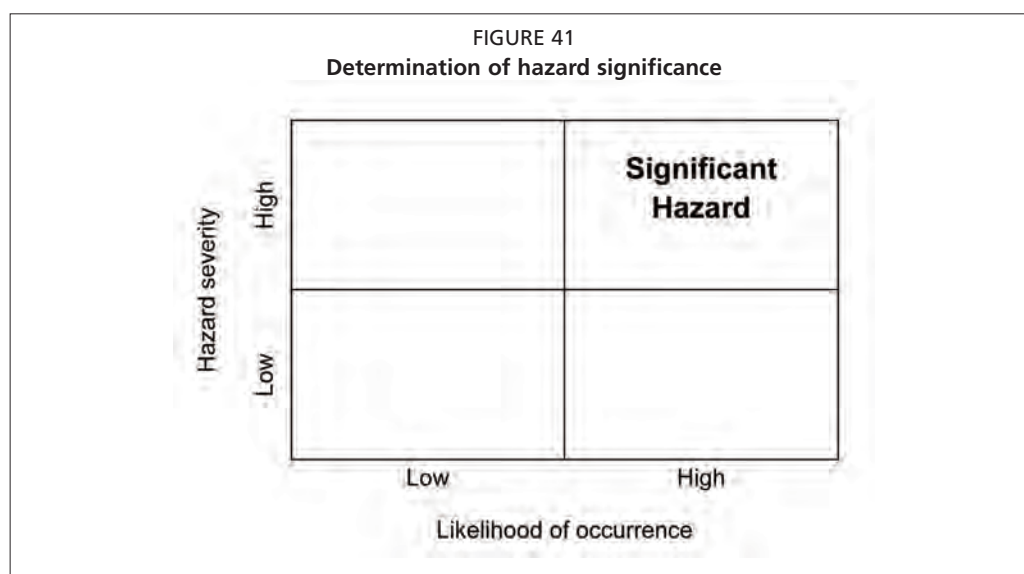
A decision tree with a number of questions can be used to determine whether potential hazards are “real”, as demonstrated in Figure 40.



Source: After ILSI (1997).

The questions in Figure 40 should be considered at each step of the processing chain, and all hazards must be considered. An element of risk assessment is involved in the evaluation of potential hazards. Only those hazards that are likely to occur and that will cause a reasonably adverse health affect are regarded as significant, as shown in Figure 41.

¹² Conditions covered by the prerequisite programme have been excluded from the list.



Source: After Mortimore and Wallace (1998).

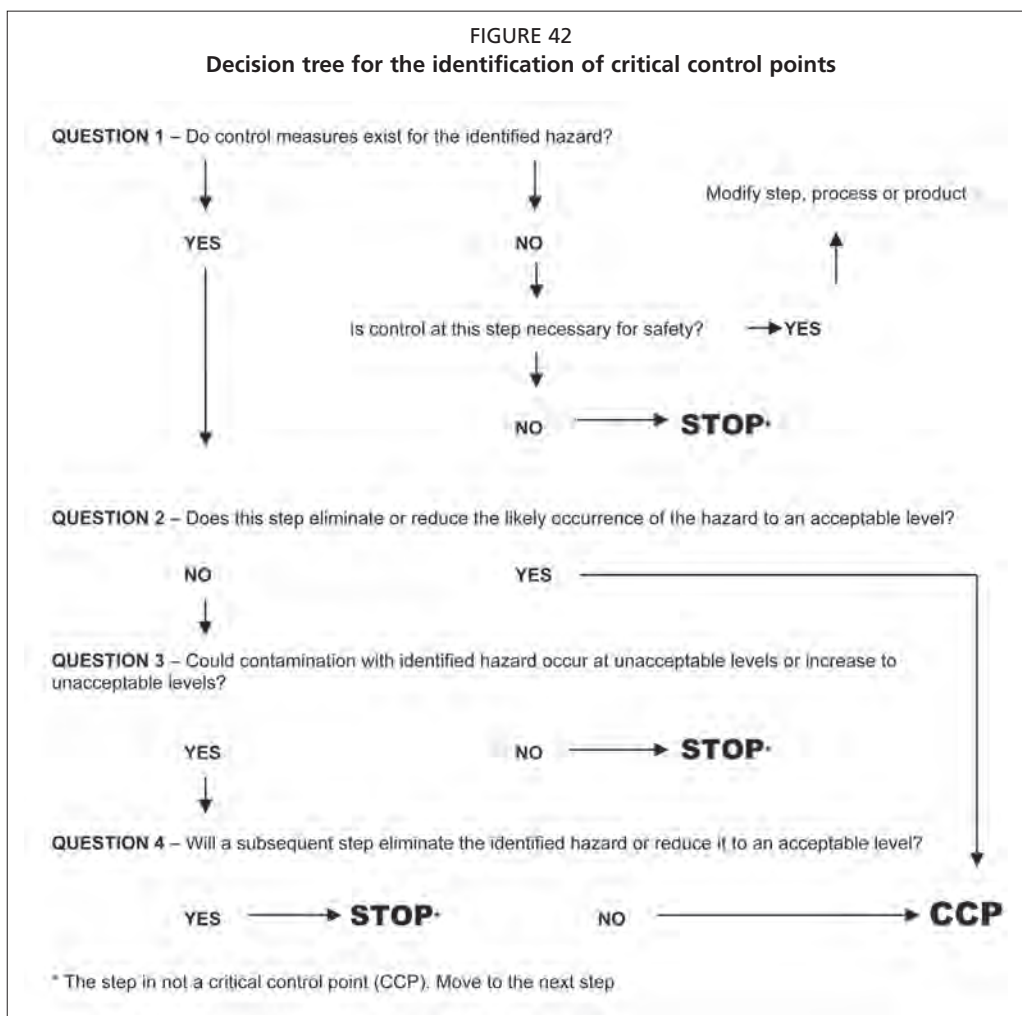
Thus, the basic procedure to use in conducting a hazard analysis is as follows:

- Based on the product description and the flow diagram, all the potential hazards associated with the product and at each production/processing step are determined and listed.
- Make a hazard evaluation:
 - assess severity of health consequences if potential hazards are not controlled;
 - determine likelihood of occurrence of potential hazards if not properly controlled.
- Using information above, determine if this potential hazard is to be addressed in the HACCP plan.
- Describe control measures.

Upon completion of the hazard analysis, the HACCP team must consider what control measures, if any, exist that can be applied for each hazard. More than one control measure may be required to control a specific hazard (or hazards) and more than one hazard may be controlled by a specific control measure. The hazards associated with each step in the production should be listed along with any measure (or measures) that is (are) used to control the hazards. A “hazard analysis worksheet” can be used to organize and document the considerations in identifying food safety hazards. An example of a hazard analysis worksheet is shown in Appendix 1.

6.3.3.7 Determine critical control points

A CCP is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. There may be more than one CCP at which control is applied to address the same hazard. Likewise, several hazards can be controlled at a single CCP. Complete and accurate identification of all the CCPs is fundamental for controlling food safety hazards. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree (Figure 42). Example of decision trees have been recommended by CAC (2003).



The application of the decision tree should be flexible depending upon the type of operation under consideration. Other approaches than the decision tree may be used for the determination of CCPs. If a hazard has been identified at a step where control is necessary for safety, and if no control measure exists at that step or at any other, then the product or the process should be modified at that step, or at an earlier or later stage, to include a control measure.

This exercise should be conducted at each step and for each hazard to identify CCPs.

6.3.3.8 Establish critical limits for each CCP

Critical limits are defined as criteria that separate acceptability from unacceptability. Critical limits represent the boundaries that are used to judge whether an operation is producing safe products as a result of proper application of the control measures. In other words, critical limits must be met to ensure that a CCP is under control.

Critical limits should be scientifically based and refer to easily measurable factors such as temperature, time, chlorine levels, water activity (a_w), pH, titratable acidity, salt concentration, available chlorine, preservatives, and sensory quality. These parameters, if maintained within boundaries, will confirm that a given hazard is under control at a given CCP. Microbiological limits, which often require days for their measurement, should be avoided by all means. However, when microbiological limits are necessary, reliable rapid microbiological techniques should be used.

The following is an example of the application of the decision tree to decide whether receiving raw material is a CCP for the presence of biotoxins and the presence of salmonella and viruses in live oysters.

Step 1: Receiving live oysters

Hazard 1: Presence of pathogenic bacteria and viruses

Control measure(s): Purchase live oysters only from a licensed harvester who has harvested them from an approved area and has tagged the containers or purchase records properly

Is step 1 a CCP for the considered hazard or not?

Question 1: Do control measures exist for the identified hazard? Yes (measures described above)

Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level? Yes. By applying the control measure described above, we avoid purchase of oysters which can not be rendered safe for human consumption by depuration

Conclusion: This step is a CCP for obtaining safe live oysters after depuration

Hazard 2: Presence of biotoxins

Control measure(s): Purchase live oysters only from a licensed harvester who has harvested them from an approved area and has tagged the containers or purchase records properly

Is step 1 a CCP for the considered hazard of biotoxins or not?

Question 1: Do control measures exist for the identified hazard? Yes (purchase only from licensed suppliers)

Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level? Yes. By using only licensed harvesters that collect only from approved areas we avoid depurating oysters containing biotoxins.

Conclusion: This step is a CCP for the considered hazard

The critical limits should meet the requirements of government regulations and/or company standards and/or be supported by other scientific data. It is essential that the persons responsible for establishing critical limits have knowledge of the process and of the legal and commercial standards required for the products. Authoritative critical limit information is available from sources such as *Fish and Fishery Products Hazards and Controls Guidance* (FDA, 2011h), other scientific publications or documents obtained from regulatory agencies, universities, expert groups or institutions.

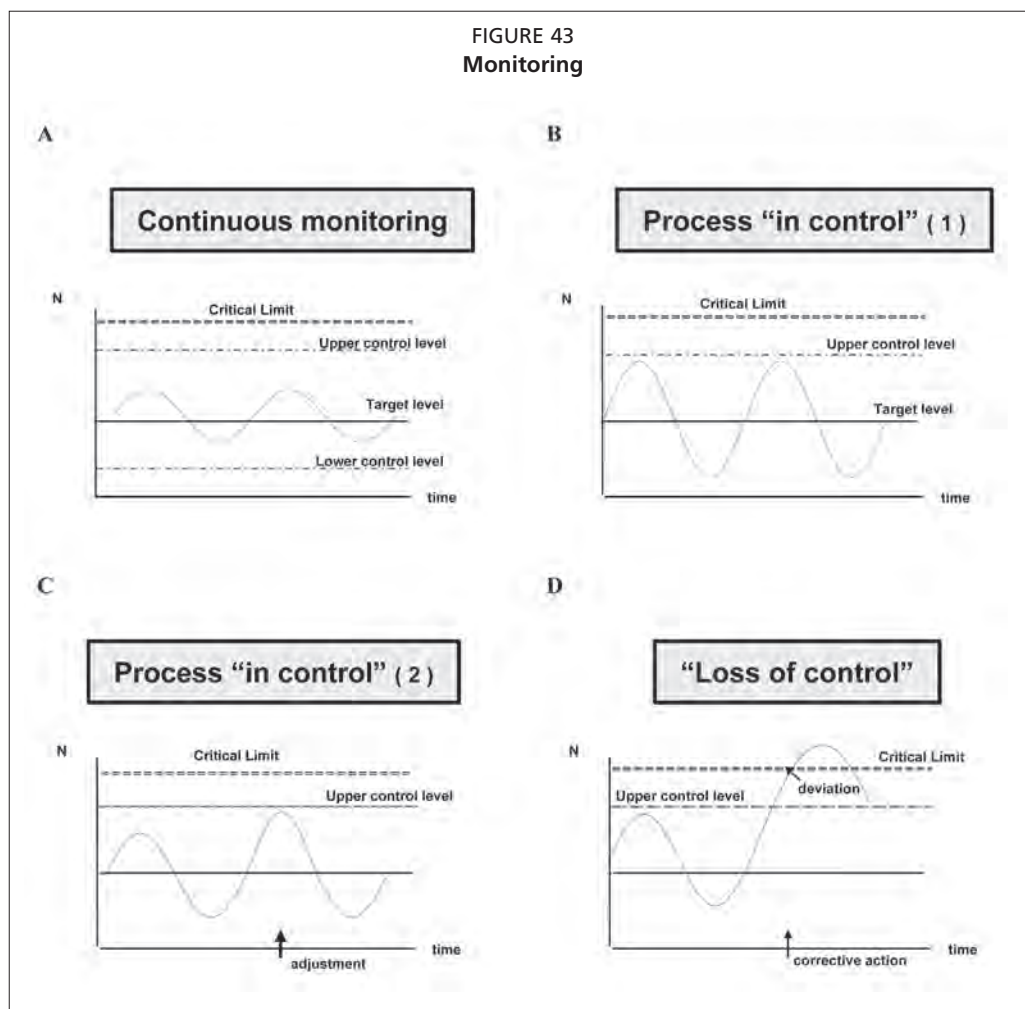
The HACCP plans provided in Appendixes 2 and 3 provide examples of critical limits for the measures designed to control the identified hazards at each identified CCP of the given processes.

6.3.3.9 Establish a monitoring system for each CCP

Monitoring is defined as the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control. The monitoring procedures will determine whether the control measures are being implemented properly and ensure that critical limits are not exceeded. The monitoring procedures must be able to detect loss of control at the CCP.

The purposes of monitoring include the following (see Figure 43):

- to measure the performance level of the system's operation at the CCP (trend analysis);
- to determine when the performance level of the system results in a loss of control at the CCP, e.g. when there is deviation from a critical limit;
- to establish records that reflect the performance level of the system's operation at the CCP to comply with the HACCP plan.



Notes: (A) small fluctuations always occur around a target level; (B) and (C) the process is under control but adjustment is needed in situation C as abnormal fluctuations are noted; (D) a deviation occurs and corrective action is needed.

Source: Motarjemi and van Schothorst (1999).

The monitoring procedures should give information on the following aspects.

What will be monitored (What?): Monitoring may mean measuring a characteristic of the process or of the product to determine compliance with a critical limit. Monitoring may also mean observing whether a control measure at a CCP is being implemented. Examples include measurement of fish temperature, sensory quality, histamine concentration, and verification of proper application of hygienic practices.

How critical limits and control measures will be monitored (How?): Deviation from a critical limit should be detected in as short a time as possible to allow prompt corrective action so as to limit the amount of adversely affected product. Again, microbiological testing is rarely effective for monitoring CCPs for this reason. Instead, physical and chemical measurements (e.g. pH, time, temperature, and sensory quality) are preferred, as they can be done rapidly and can often be related to the microbiological control of the process. This correlation between rapid measurements and microbiological control needs to be regularly validated.

Equipment used for monitoring procedures should undergo periodic calibration or standardization as necessary to ensure accuracy.

Operators should be trained in the proper use of the monitoring equipment and should be provided with a clear description of how the monitoring should be carried out.

Frequency of monitoring (When?): Wherever possible, continuous monitoring is preferred. Continuous monitoring is possible for many types of physical or chemical methods. Examples of continuous monitoring would include the automatic measurement of free chlorine levels in water, time and temperature of sterilization, and freezing temperature.

Where non-continuous monitoring is the chosen system, the frequency of monitoring should be determined from historical knowledge of the process and product. If a problem is detected, the frequency of monitoring may need to be increased until the cause of the problem is corrected.

Who will monitor (Who?): Careful consideration should be given to assigning responsibility for monitoring. Once assigned, the individual responsible for monitoring a CCP must:

- be adequately trained in the CCP monitoring techniques;
- fully understand the importance of the CCP monitoring techniques;
- have ready access (be close) to the monitoring activity;
- accurately report each monitoring activity;
- have the authority to take appropriate action as defined in the HACCP plan;
- immediately report critical limit deviation to supervisor.

Where to monitor (Where?): Monitoring takes place at each CCP where a given control measure is applied to control a given hazard.

The HACCP plans provided in Appendixes 2 and 3 indicate the monitoring procedures recommended for various seafood operations.

6.3.3.10 Establish corrective actions

As the main reason for implementing HACCP is to prevent problems from occurring, corrective actions should be predefined and taken when the results of monitoring at the CCP indicate a loss of control. Loss of control can cause a deviation from a critical limit for a CCP. All deviations must be controlled by taking predetermined actions to control the non-compliant product and to correct the cause of non-compliance.

Product control includes proper identification, control and disposition of the affected product. The establishment should have effective procedures in place to identify, isolate (separate), mark clearly and control all products produced during the deviation period.

Corrective action procedures are necessary to determine the cause of the problem, take action to prevent recurrence and follow up with monitoring and reassessment to ensure that the action taken is effective. Reassessment of the hazard analysis or modification of the HACCP plan may be necessary to eliminate further recurrence.

The control and disposition of the affected product and the corrective actions taken must be recorded and filed. Records should be available to demonstrate the control of products affected by the deviation and the corrective action taken. Adequate records permit verification that the establishment has deviations under control and has taken corrective action.

The HACCP plans given in Appendixes 2 and 3 provide examples of corrective actions recommended for various seafood operations.

6.3.3.11 Establish verification procedures

Verification is the application of methods, procedures and tests, including random sampling and analysis and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan. The objective of verification procedures is to determine whether the HACCP system is working effectively.

Careful preparation and implementation of the HACCP plan does not guarantee the plan's effectiveness. Verification procedures are necessary to assess the effectiveness of the plan and to confirm that the HACCP system adheres to the plan.

Verification should be undertaken by an appropriately qualified individual (or individuals) capable of detecting deficiencies in the plan or its implementation.

Verification activities should be documented in the HACCP plan. Records should be made of the results of all verification activities. Records should include methods, date, individuals and/or organizations responsible, results or findings and actions taken.

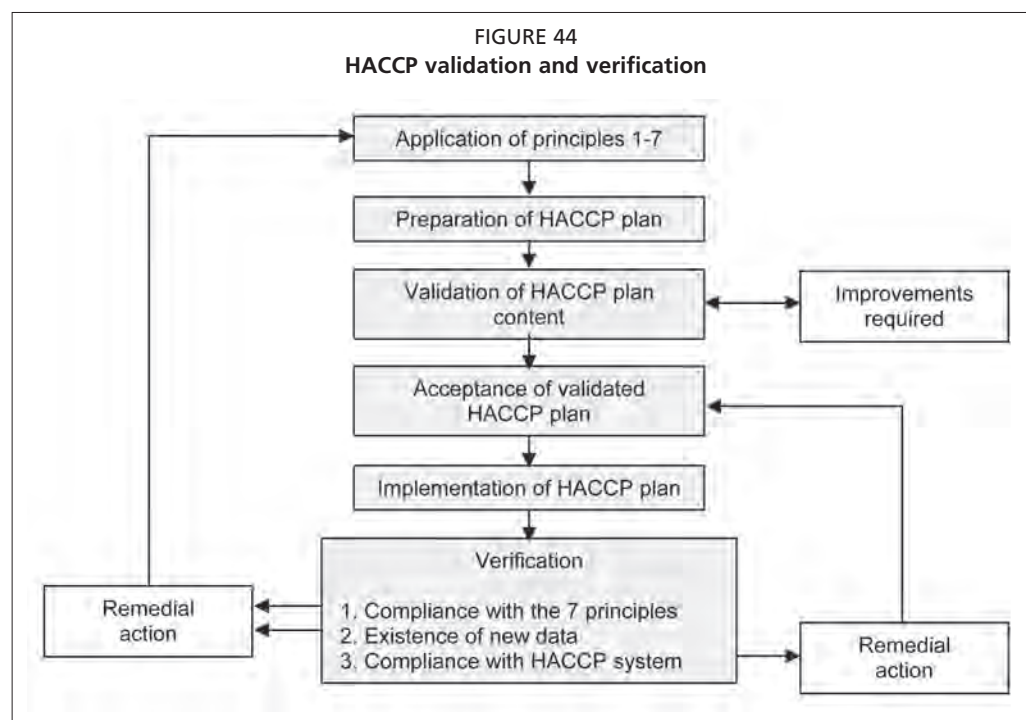
For example, the following verification procedure can be recommended:

Wherever needed but at least weekly, the HACCP team assesses internally all the results of the controls, monitoring and corrective actions and draws conclusions for the subsequent production weeks.

On a longer term, bi-annually or annually for example, the HACCP team can:

- Evaluate the monitoring and corrective actions data to assess performance and analyse the reasons for any loss of control or for complaints from clients and/or control authorities.
- Use the results of this analysis to update the HACCP manual, identify further training and improved practices, performance or maintenance, modify frequency (increase or decrease) of specific monitoring and revise the list of approved suppliers.
- Perform an audit by an external specialist to assess the performance of each control, monitoring or corrective procedure. He/She will examine the different records, including records for monitoring, calibration and maintenance, training, complaints and reports from clients and control authorities. He/she will prepare a report that will be submitted to management and discussed during a meeting with management and the HACCP team. The audit exercise will be also used as an opportunity to introduce new procedures, monitoring techniques or critical limits to take into consideration new developments, including new regulatory requirements.

Apart from the initial validation, subsequent validation as well as verification must take place whenever there is a change in raw materials, product formulation, processing procedures, consumer and handling practices, new information on hazards and their control, consumer complaints, recurring deviations or any other indication, that the system is not working. Figure 44 shows where validation fits into the process of HACCP implementation.



Source: After ILSI (1999).

6.3.3.12 Establish documentation and record-keeping

Records and documentation are essential for reviewing the adequacy of and adherence to the HACCP plan. Several types of records should be considered among those relevant in an HACCP programme:

- support documentation, including validation records, for developing the HACCP plan;
- records generated by the HACCP system: monitoring records of all CCPs;
- deviation and corrective action records, verification/validation records;
- documentation on methods and procedures used;
- records of employee training programmes.

Records may be in different forms, e.g. processing charts, written procedures or records, and tables. They can be stored in paper or electronic forms, provided that assurance of record integrity is provided. It is imperative to maintain complete, current, properly filed and accurate records. Failure to document the control of a CCP or implementation of a corrective action would be a critical departure from the HACCP plan.

6.4 APPLICATION OF HACCP PRINCIPLES IN THE FISH AND AQUACULTURE INDUSTRY (LAHSEN ABABOUCHE)

As mentioned above, hazard analysis, including the identification of adequate control measures, establishment of critical limits and monitoring procedure are the three most science-based principles of HACCP. They require a good background in food science,

food microbiology and food safety. The other elements of HACCP plan development and application require training and practical experience.

The following section provides the necessary scientific background information to enable readers to perform proper hazard analysis. Section 6.4.13 is an example illustrating how a HACCP plan can be developed in the fish canning industry. Another example is provided in Section 6.10.4 to illustrate the applicability of HACCP in aquaculture. These two examples are provided for illustrative or training purposes. They should not be used, under any circumstances, for similar fish and seafood operations without adaptation and validation by an HACCP team.

6.4.1 Considerations for the development of HACCP plans in fisheries and aquaculture

The safety of seafood products varies considerably and is influenced by a number of factors such as origin of the fish, microbiological ecology of the product, handling and processing practices and preparations before consumption. Taking most of these aspects into consideration, seafood can conveniently be grouped as shown below (modified from Huss, 1994):

- Molluscan shellfish.
- Raw fish to be eaten without any cooking.
- Fresh or frozen fish and crustaceans – to be fully cooked before consumption.
- Lightly preserved fish products, i.e. NaCl < 6 percent in water phase, pH > 5.0. The prescribed storage temperature is < 5 °C. This group includes salted, marinated, cold-smoked and gravad fish.
- Fermented fish, i.e. NaCl < 8 percent NaCl, pH changing from neutral to acid. Typically, the products are stored at ambient temperature.
- Semi-preserved fish, i.e. NaCl > 6 percent in water phase, or pH < 5, preservatives (sorbate, benzoate, nitrite) may be added. The prescribed storage temperature is < 10 °C. This group includes salted and/or marinated fish or caviar, fermented fish (after completion of fermentation).
- Mildly heat-processed (pasteurized, cooked, hot-smoked) fish products and crustaceans (including precooked, breaded fillets). The prescribed storage temperature is < 5 °C.
- Heat-processed (sterilized, packed in sealed containers).
- Dried, smoke-dried fish, heavily salted fish. Can be stored at ambient temperatures.

However, the safety of seafood products and processing cannot be studied in isolation. A large number of hazards are related to the pre-harvest situation or raw-material handling and must be under control when the raw material is received at the processing factory.

6.4.2 Hazard analysis of raw material

Most fish and shellfish are still extracted from a wild population, but aquaculture is a fast-growing food production system (as outlined in Section 1.2.1). While there are specific safety aspects associated with wild fish caught in the high seas, the intensive husbandry in aquaculture poses new and increased risks. It is imperative that the HACCP principles are extended beyond the factory-gate and applied throughout the entire food production chain from harvest to the consumers' plate.

In a general hazard analysis of the pre-harvest conditions for fish and shellfish and the procedures for handling the raw material before it is received at the processing plant, a number of significant hazards can be identified:

6.4.2.1 *Pathogenic bacteria*

Pathogenic bacteria from the aquatic or general environment may be present in low numbers in all fish and shellfish at the time of harvest (see Section 3.2.1). This is not a significant hazard as it is unlikely that these pathogens will be there in sufficient numbers to cause disease – even if the fish are eaten raw. However, if growth and toxin production of these organisms is taking place as a result of time/temperature abuse, it is reasonably likely that these pathogens and their toxins could reach unsafe levels. For fish to be eaten raw or used as raw material in products that are not heat-treated, this situation is a significant hazard that must be controlled. High numbers of pathogenic *Vibrio* spp. may accumulate in bivalves, but it is unlikely that pathogenic levels will be reached.

Pathogenic bacteria from animal/human reservoir may be present in fish and shellfish harvested in contaminated waters. This is a significant hazard for fish and shellfish to be eaten raw owing to the low minimum infective dose for some of these organisms.

The preventive measures for these hazards are control and monitoring of harvest areas for faecal pollution and placing a limit on the time between harvest and refrigeration to prevent growth and toxin production.

6.4.2.2 *Viruses*

The presence of viruses in the harvest area is of particular concern in molluscan shellfish because:

- environments where molluscan shellfish grow are often subject to contamination from sewage, which may contain pathogens (bacteria, viruses);
- molluscan shellfish filter and concentrate pathogens that may be present in the water;
- molluscan shellfish are often consumed raw or only partially cooked.

Thus, the presence of a virus is a significant hazard in molluscan shellfish and fish to be eaten raw. The preventive measure is control and monitoring of harvesting areas for faecal pollution (see Section 3.2.3).

6.4.2.3 *Biotoxins*

Contamination of fish and shellfish with natural toxins from the harvest area can cause serious illness in consumers. The toxins accumulate in fish when they feed on marine algae, where the toxins are produced. They occur in fish from the tropical and subtropical areas (ciguatera) and in shellfish worldwide (see Section 3.2.5). In order to determine whether CFP is a significant hazard, some guidance can be provided by the historical occurrence of the toxin and knowledge about the safety of the reefs from which the fish has been obtained.

The preventive measures for the presence of toxins in shellfish are control and classification of shellfish-harvesting areas. As a result, shellfish harvesting is only allowed from “safe” waters. A significant element in this system is the requirement that all shellfish containers bear a tag that identifies the type and quantity of shellfish, the harvester, harvest location and date of harvest.

The preventive measure for CFP is to ensure that incoming fish have not been caught in an area for which there is a CFP advisory or for which there is knowledge that CFP is a problem.

6.4.2.4 *Biogenic amines*

These amines are produced as a result of time/temperature abuse of certain fish species and they can cause illness in consumers (see Section 3.2.2). It is therefore a post-harvest

hazard, but very often it is a pre-receiving hazard introduced during handling on board the fishing vessel or during transportation to the plant after landing.

The preventive measure is rapid chilling of fish immediately after capture. Generally, fish should be packed in ice or chilled seawater within 12 h after catch or – in case of large fish such as tuna – chilled to an internal temperature of 10 °C or less within 6 h after capture.

6.4.2.5 Parasites

It is reasonably likely that parasites will be present in significant numbers of certain wild-caught fish species – and certain aquaculture fish if they are fed on unheated processing waste or bycatch fish (see Section 3.2.4). Therefore, parasites should be considered a significant hazard, and a preventive measure to eliminate parasites must be identified during processing of particular fish products.

6.4.2.6 Chemicals

Concerns about this hazard primarily focus on fish harvested from freshwater, estuaries and near-shore coastal waters and on fish from aquaculture. Without proper control, it would be reasonable to expect that unsafe levels of chemicals could be present in the fish, thus representing a significant hazard.

The preventive measure is the presence of government-controlled monitoring programmes and ensuring that fish or shellfish have not been harvested from waters closed to commercial fishing. For aquaculture, the preventive measures are full control of chemical contamination of the environment (soil/water) surrounding the aquaculture site, control of water quality and control of the feed supply. Only approved agrochemicals and veterinary drugs should be used and only according to manufacturers' instructions. Correct withdrawal of veterinary drugs must be observed.

Table 64 summarizes the hazard analysis of the pre-harvest/pre-receiving step for all hazards.

TABLE 64
Hazard analysis of pre-harvest conditions and raw material handling

Organism / component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria:								
indigenous	–	+	high	high	+	–	–	+
non-indigenous	+	+	high	high	+	+	+	+
Viruses	+	–	high	high/low ²	+/-	+	+	+
Biotoxins	+	–	high	high/low ²	+/-	+	–	+
Biogenic amines	–	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	high	+	–	–	+
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

One of the major problems in ensuring the safety of seafood products is that processors often have no control and only limited information about the history of the raw material. This is a serious weakness, and every effort must be made to overcome this problem. The significant hazards associated with the raw material must be identified and controlled before the raw material is received at the factory. The receiving step is the first CCP in any seafood processing, and the monitoring procedures will mainly be to check documents (certificates of origin, harvester, date and location of harvesting, copies and results of government monitoring programmes, etc.).

6.4.3 Molluscan shellfish

Molluscan shellfish are harvested by being raked or trawled from the bottom (oysters and mussels) or dug from the sand at low tide (clams and cockles). After harvesting, the shellfish are sorted (by size), washed and packed in bags or crates or left in a pile on deck. The shellfish may be transported and sold live to the consumer or they may be processed (shucked) raw or by use of heat. The heat applied in processing is only enough to facilitate shucking by causing the animal to relax the adductor muscle, and it has no effect on the microbial contamination of the animals. The shucked meat is washed, packed and sold fresh, frozen or further processed and canned.

Most molluscs (oysters, mussels, clams and cockles) grow and are harvested in shallow, near-shore estuarine waters. Thus, there is a strong possibility that the live animals may be contaminated with sewage-derived pathogens (pathogenic bacteria, and viruses) as well as those from the general environment. In addition, biotoxins and chemicals can be present. Owing to the filter feeding of molluscs, a high concentration of disease agents may be present in the shellfish and this, therefore, constitutes a serious hazard. During processing, further contamination with pathogens (bacteria and viruses) may occur, including the growth of bacteria if time and temperature conditions are favourable. As most molluscs are traditionally eaten raw or very lightly cooked, this will further increase the risk. Thus, a number of significant hazards can be identified in molluscan shellfish, as shown in Table 65.

TABLE 65
Hazard analysis of processing of bivalve shellfish

Organism / component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria:								
indigenous	+	+	high	high	+	–	–	+
non-indigenous	+	+	high	high	+	+	+	+
Viruses	+	–	high	high	+	+	+	+
Biotoxins	+	–	high	high	+	+	–	+
Biogenic amines	–	–						
Parasites	–	–						
Chemicals	+	–	medium	high	+	+	–	+

¹ PP = prerequisite programme

It follows that the significant hazards to be controlled in molluscan processing are:

- contamination with pathogens (bacteria, viruses), biotoxins and chemicals from the harvesting area;
- further contamination with pathogens (bacteria and viruses) during processing;
- growth of pathogens during processing and storage.

The following preventive measures can be applied to minimize the risks outlined above:

- Regarding (a)
- Control and monitoring of harvesting areas. Check for tags and ensure that incoming raw material is from licensed harvesters or certified dealers.
 - Depuration.

It is well known that none of these measures are 100 percent effective, but no other CCP can be identified for this hazard (contamination). For this reason, molluscs to be eaten raw should be provided with a warning label to inform consumers of the risk.

- Regarding (b) • Further contamination during processing is a hazard, which will be controlled by the prerequisite programme.
- Regarding (c) • Limit the time from harvest to refrigeration.
- Proper chilling (< 5°C) at all times during storage (raw material and final product). This aspect is included in the prerequisite programme.

Therefore, the only two CCPs to be identified and included in the HACCP plan are: (i) the receiving step, where it is possible to exercise control of the source of the molluscs; and (ii) the labelling step, where it can be checked that the warning on the consumption of raw molluscs is on the label. The following details could be entered in the HACCP plan for the receiving step:

- | | |
|----------------------|--|
| Critical limits | <ul style="list-style-type: none"> • All shell stock containers must bear a tag that discloses the date and place where harvested, the quantity and name and licence number of harvester. No molluscs from closed areas must enter the plant. |
| Monitoring programme | <ul style="list-style-type: none"> • What: tags, labels, licence of fisher. • How: visual check. • When: all containers. • Who: receiving employee, supervisor or quality control (QC) staff. |
| Corrective actions | <ul style="list-style-type: none"> • Reject if untagged or from closed areas. |
| Record-keeping | <ul style="list-style-type: none"> • Receiving records on all shellfish (quantity, harvesting details). |
| Verification | <ul style="list-style-type: none"> • Daily review of records. |

6.4.4 Raw fish – to be consumed raw

The hazards related to these products are primarily associated with the pre-harvest/pre-receiving situation. However, in the hazard analysis, some of these hazards can be excluded. As stated above, contamination of raw fish with indigenous pathogenic bacteria is unlikely to be high enough to provoke disease and, therefore, it is not a significant hazard. Growth of these bacteria and of HPB is a potential hazard, but it is very unlikely in a product to be eaten raw. For this to happen, the fish must be kept for some time at elevated temperatures and, in this case, also spoilage organisms will grow. As the latter will grow much faster than the pathogens, the fish is likely to spoil or be unfit for raw consumption before sufficient growth of pathogens and HPB has taken place. The results of a general hazard analysis are shown in Table 66.

The significant hazards are:

- a) Contamination of fish with non-indigenous bacteria, viruses, biotoxins or environmental chemical contaminants (heavy metals, pesticides, and veterinary drugs in aquaculture).
- b) Presence of parasites.

TABLE 66

Hazard analysis of raw fish to be consumed raw

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	–	+	high	low	–			
non-indigenous	+	–	high	high	+	+	+	+
Viruses	+	–	high	high	+	+	+	+
Biotoxins	+	–	high	high/low ²	+/-	(+)	–	+
Biogenic amines	–	+	low	low	–			
Parasites	+	–	low	high	+	–	–	+
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

The following preventive measures can be applied:

- Regarding (a)
- Control and monitoring of harvesting areas including control of the use of drugs in aquaculture.
 - Contamination (bacteria and viruses) during processing is controlled by the prerequisite programme.
 - Prohibition of the use of puffer fish for human consumption.
 - Avoidance (sorting) of fish with a record of causing ciguatera.
- Regarding (b)
- Introduction of a freezing step to eliminate the risk from parasites.

While the preventive measure for control of parasites is 100 percent effective, this is not the case for control of the pre-harvest contamination of fish with pathogenic organisms or compounds. There are serious weaknesses in a monitoring programme, and no effective CCP can be identified for the control of ciguatera.

Only two CCPs are identified in the processing of raw fish to be eaten raw:

- the receiving step;
- the freezing step.

- Critical limits
- In situations where contamination with non-indigenous pathogens from the harvest area as well as contamination with any chemical is a possibility, a source control or certificate must accompany all lots of fish. This certificate must ensure that the fish were not harvested in waters that are closed to fishing or in any way contaminated with unwanted compounds (i.e. drugs in aquaculture fish).

- Monitoring programme
- What: time and temperature at freezing step. Tags, labels, licence of fisher.
 - How: visual check.
 - When: all containers. Continuous recording of freezing temperature.
 - Who: receiving employee, supervisor or QC staff.

Corrective actions	<ul style="list-style-type: none"> • Reject if untagged or from closed areas. • Adjust freezer. Refreeze material not properly frozen.
Record-keeping	<ul style="list-style-type: none"> • Receiving records on all fish raw material (quantity, harvesting details). • Temperature records.
Verification	<ul style="list-style-type: none"> • Daily review of records.

6.4.5 Fresh/frozen fish and crustaceans – to be fully cooked before consumption

The hazard analysis of these products is relatively straightforward and uncomplicated. In most cases, the animals are caught in the sea or freshwater, handled and processed without any use of additives or chemical preservatives, and finally distributed as chilled or frozen products.

The epidemiological evidence has shown that the presence of histamine or biotoxins accounts for almost 80 percent of all disease outbreaks caused by “fish”. Low levels of pathogenic bacteria and viruses may be present on raw fish as part of the natural flora and/or as a result of contamination during handling and processing. As the product will be cooked before consumption, it is very unlikely that this low level of pathogens will cause any disease. Even if any growth has taken place in the raw fish to be cooked, it is unlikely to produce any disease. Therefore, pathogenic bacteria and viruses are not significant hazards that need to be controlled.

In contrast, biotoxins (ciguatoxin and tetrodotoxin) are heat stable, and cooking the fish before consumption is not likely to eliminate this hazard. In areas where this hazard is likely to occur (see Section 3.2.5.6), it must be noted as a significant hazard.

Similarly, biogenic amines (histamine) are resistant to heat and, if present in the raw fish, are likely to cause poisoning. Therefore, production of histamine in raw fish is a significant hazard that must be controlled (see Section 3.2.2).

Parasites are common in fish, but normal household cooking will kill the parasites. Therefore, their possible presence is not a significant hazard.

Chemical contamination of fish is unlikely and not a significant hazard except for aquaculture fish and fish from coastal areas subject to industrial pollution (see Section 3.3.2). Table 67 summarizes the hazard analysis for this product.

TABLE 67

Hazard analysis of fresh/frozen fish and crustaceans to be cooked before consumption

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	–	+	high	low	–			
non-indigenous	+	+	high	low	–			
Viruses	+	–	high	low	–			
Biotoxins	+	–	high	high/low ²	+/-	+	–	+
Biogenic amines	–	+	low	high/low ²	+/-	–	+	+
Parasites	+	–	low	low	–			
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

Thus, the significant safety hazards are:

- Presence of biotoxins. This hazard only applies to fish from warm waters with a history of causing ciguatera (CFP) and to puffer fish.
- Formation of histamine. This hazard mainly applies to scombroid fishes.
- Presence of chemicals. This hazard only applies to fish from aquaculture or coastal areas.

For all other fish (the large majority of marine fish) caught in clean waters and sold as fresh or frozen fish and fish products, there are likely to be no safety hazards and thus no HACCP plan will result. However, a hazard analysis worksheet needs to be completed and prerequisite programmes properly implemented.

The preventive measures that can be applied to the significant hazards are:

- Sorting of the catch to exclude puffer fish. Making sure that the fish have not been caught in an area for which there is a CFP advisory or for which there is knowledge of a CFP problem. Although the latter preventive measure is not 100 percent effective, no other means are available.
- Rapid chilling of fish immediately after catch to temperatures $< 10^{\circ}\text{C}$ is the most important element in any strategy for preventing the formation of histamine. Further chilling towards the freezing point is desirable to prevent long-term low-temperature development of histamine. Control of temperature is part of the prerequisite programme
- The preventive measure for chemical contamination of fish is to compare information on the capture area with government bans on fishing.

Based on the above, the only CCP for raw fish to be cooked before consumption is the receiving step (possible histamine formation during processing and storage of scombroid fish is taken care of by the prerequisite programmes). The following details can be entered in the HACCP plan:

Critical limits	<ul style="list-style-type: none"> • No puffer fish allowed in processing. No fish from an area where there is an CFP advisory is allowed in processing. • No fish harvested in an area closed for fishing is allowed in processing. • For histamine the critical limit is < 50 ppm.
Monitoring programme	<ul style="list-style-type: none"> • What: Sorting procedures, tags, labels, harvesting vessels record decomposition of lot. Temperature records. • How: Visual check. • When: All lots. • Who: Receiving employee.
Corrective actions	<ul style="list-style-type: none"> • Reject lots with no information on catching area, or if from closed area. • Reject the lot or perform histamine analysis on lots of poor sensory quality. • Inform harvester, adjust cooling procedures.
Record-keeping	<ul style="list-style-type: none"> • Receiving records, all lots, temperature records.
Verification	<ul style="list-style-type: none"> • Records review, calibration of thermo-recorders, histamine analysis of selected samples.

6.4.6 Lightly preserved fish product

This group includes fish products with low salt content (water phase salt [WPS] < 6 percent) and low acid content (pH > 5.0). Preservatives (sorbate, benzoate, nitrogen dioxide and smoke) may or may not be added. The products may be prepared from raw or cooked raw material, but are normally consumed without any prior heating. Product examples are salted, marinated, cold-smoked or gravad fish. These products have a limited shelf-life and are typically stored at temperature < 5 °C.

The presence in these products of low numbers of pathogenic bacteria normally found in the aquatic and the general environment (*Clostridium botulinum*, pathogenic *Vibrio* sp., and *Listeria monocytogenes*) is a potential hazard. Owing to their low numbers, the mere presence is not a significant hazard. However, if these organisms are allowed to grow to high numbers, they are very likely to cause a serious disease, and therefore, they represent a significant hazard. It should be remembered that growth and toxin production can take place in the raw material as well as in the final product.

Contamination of products during processing with viruses and non-indigenous pathogenic bacteria, as well as possible growth of the latter, are also potential hazards. However, these hazards are prevented by the prerequisite programme and, therefore, not likely to occur.

The presence of biotoxins (CFP) is a potential hazard if the raw material is a fish species with a history of causing CFP and originating in an area where CFP is known to occur.

Production of biogenic amines is a significant hazard in all products based on scombroid fish or all fish containing large amounts of free histidine in the flesh. The production requires growth of histamine-decarboxylating bacteria. A number of different bacteria are able to produce histamine under various conditions (as discussed in Section 3.2.2). It should be remembered that biogenic amines may be produced in the raw material as well as in final products.

Parasites are common in many fish species in all parts of the world, and the processing conditions and preservative parameters for lightly preserved fish products are not sufficient to kill the parasites. Thus, a “processing for safety” step must be included in the process of these types of products to control this significant hazard.

Chemical contamination of raw material is a potential hazard if it originates in aquaculture or certain coastal fisheries. Only if this is the case, should chemical contamination be regarded as a significant hazard. The hazard analysis is summarized in Table 68.

TABLE 68
Hazard analysis of lightly preserved fish products

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	–	+	high	high	+	–	–	+
non-indigenous	+	+	high	high	+	–	+	–
Viruses	+	–	high	high	+	–	+	–
Biotoxins	+	–	high	high/low ²	+/-	+	–	+
Biogenic amines	–	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	high	+	–	–	+
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

The significant hazards are the result of:

- a) growth of pathogenic bacteria from the aquatic or the general environment;
- b) production of biogenic amines (scombroid fish);
- c) presence of parasites;
- d) chemical contamination (depending on geographical area).

The following preventive measures can be applied:

- Regarding (a) • Growth of *C. botulinum* can be prevented by WPS > 3.5 percent and a storage temperature < 5 °C.
- Growth of *L. monocytogenes* cannot with certainty be prevented by the parameters used in the preservation of this category of products.
- An alternative solution is to reduce shelf-life of the products to a period of no growth of *L. monocytogenes*. The length of this period needs to be established by experimentation.
- Regarding (b) • Storage at low temperature (< 5 °C) will prevent the growth of a number but not all of HPB. There are no experimental data to demonstrate complete control of this hazard.
- Regarding (c) • Introduction of a freezing step (–20 °C for at least 24 h).
- Regarding (d) • Securing raw material from areas with no chemical contamination.

Based on the considerations above, the following CCPs can be identified: Receiving step, salting step and freezing step.

The following details can be entered in the HACCP plan:

- | | |
|----------------------|---|
| Critical limits | <ul style="list-style-type: none"> • Receiving step: only raw material of good sensory quality will be used. No fish from an area where there is a CFP advisory must be used. No fish harvested in area closed for fishing is allowed. • Salting: WPS ≥ 3.5 percent NaCl. • Freezing step: –20 °C for at least 24 h. • Storage temperature: ≤ 5 °C. |
| Monitoring programme | <ul style="list-style-type: none"> • What: sensory quality of raw material. Certificate of origin of fish. Salting procedures. Temperatures and times of freezing. • How: visual. • When: all lots. Continuous recording of temperature. • Who: receiving employee. QC staff. |
| Corrective actions | <ul style="list-style-type: none"> • Reject lots of poor quality or with no certificate of origin. • Adjust salting process. • Check WPS in lots produced when process is out of control. • Adjust freezing procedures. |

6.4.7 Fermented fish

Traditionally the term “fermented fish” covers both enzyme hydrolysed and microbial fermented fish products. However, a clear distinction should be made between these products. Thus, Paludan-Müller (2002) suggests defining fermented fish as “products which contain a carbohydrate source and in which the level of salt is less than 8 percent water phase salt (WPS)”. This level of salt (< 8 percent) allows the fermentative growth of lactic-acid bacteria and a concomitant decrease in pH to < 4.5. In contrast, enzyme hydrolysed fish has a WPS > 8 percent and a final pH of 5–7. A large number of different fermented fish products are found in Southeast Asia. The products are traditionally stored at ambient temperatures and consumed without any cooking. Fermented fish products have been associated with a number of outbreaks of food-borne diseases such as botulism, trematodiasis, salmonellosis and vibriosis.

The natural presence of pathogenic bacteria from the aquatic and general environment is not considered a significant hazard in this product owing to the low numbers. However, conditions for growth of some of these organisms (*C. botulinum* type A and B, *Listeria monocytogenes*, and *Vibrio* sp.) are good until the pH decreases to almost 4.5. This takes about 1–2 days at 30 °C in a natural fermentation. Therefore, rapid and adequate acidification is the preventive measure for this significant hazard. For complete safety, temperatures during fermentation should be kept at < 10 °C until the final pH has been reached.

Contamination of fermented fish products with pathogenic bacteria from the animal/human reservoir and with pathogenic viruses are potential hazards, which will be controlled by the prerequisite programme.

Most fermented fish products are based on freshwater fish as raw material. However, if marine fish are used, the presence of biotoxin (ciguatera) should be considered a potential hazard (as discussed in Section 3.2.5.6). Formation of biogenic amines (histamine) is a health hazard primarily related to marine, scombroid fish species and is not a potential hazard when freshwater fish are used as raw material.

Parasites, particularly trematodes, are very common in fish used as raw material for fermented fish. As there is no killing step for these parasites in the normal processing, they are very likely to cause disease and must be regarded as a significant hazard. The preventive measures are food safety education and to bring about changes in the traditional consumption practices of eating non-cooked fermented fish. Until then, fermented fish that is to be eaten without any cooking must have a freezing step included. The concerns for chemical hazards are related to the raw material. The hazard analysis for fermented fish products is summarized in Table 69.

TABLE 69
Hazard analysis of fermented fish

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	–	+	high	high	+	–	–	+
non-indigenous	+	+	high	high	+	–	+	–
Viruses	+	–	high	high	+	–	+	–
Biotoxins	+	–	high	high/low ²	+/-	(+)	–	+
Biogenic amines	–	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	high	+	–	–	+
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

The CCPs in production of fermented fish are:

- | | |
|--|---|
| Receiving step: | • Check raw materials. |
| Time/temperature conditions during fermentation: | • Inhibition of growth of indigenous pathogens. |
| Freezing step: | • Control of parasites. |

6.4.8 Semi-preserved fish

These are fish products with > 6 percent WPS or a pH < 5.0. Preservatives (sorbate, benzoate and nitrate) may or may not be added. These products require chill storage (< 10 °C) and may have a shelf-life of six months or more. Normally, there is no heat-treatment applied during processing or in the preparation before consumption. Traditional production often includes a long ripening period (several months) of the raw material before final processing. Product examples are salted and marinated fish, fermented fish and caviar products.

There is epidemiological evidence that these types of products have been the cause of illness related to the presence of bacterial toxins (botulism), parasites, biotoxins and histamine.

The presence of low numbers of pathogenic bacteria normally found in the environment is not a significant hazard in these products (not likely to cause disease). Contamination with non-indigenous pathogens (bacteria and viruses) is a potential hazard to be prevented by the prerequisite programme.

Growth and possible toxin production of pathogenic bacteria is not possible in these products if they are correctly processed and the storage temperature is kept at < 10 °C. As with lightly preserved fish products, it must be pointed out that growth and toxin production may take place in the raw material. Bacterial toxins, including botulinum toxins are very stable at high salt and low pH (Huss and Rye Petersen, 1980). Any toxin present or preformed in the raw material will be carried over to the final product, and this hazard can only be controlled by having full control over the complete handling and processing steps from harvesting to consumption.

Biotoxins (ciguatera) is a potential hazard only if the raw material used is a fish species with a history of causing CFP and originating in an area where CFP is known to occur. This is not very likely to happen, and, therefore, biotoxins are not a significant hazard for this product.

Production of biogenic amines may take place both in the raw material and in the final product. It is a significant hazard as it is very likely to occur in scombroid fish if there is a loss of control.

Parasites are very common in fish species used as raw material for semi-preserved products. Therefore, this hazard is significant (likely to occur) and must be prevented.

Chemical contamination of raw material is a potential hazard if it originates from aquaculture or certain coastal fisheries. Table 70 summarizes the hazard analysis of these products.

TABLE 70

Hazard analysis of semi-preserved fish

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	–	+	high	high	+	–	–	+
non-indigenous	+	+	high	high	+	–	+	–
Viruses	+	–	high	high	+	–	+	–
Biotoxins	–	–						
Biogenic amines	–	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	high	+	–	–	+
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

The CCPs in production of semi-preserved fish products are:

- | | |
|---|---|
| Receiving step: | <ul style="list-style-type: none"> • Check raw material. |
| Time/temperature conditions: | <ul style="list-style-type: none"> • Chilled storage for prevention of growth of pathogens. Critical limits are: • < 5 °C for raw materials; • < 10 °C for final products. |
| Salting step: | <ul style="list-style-type: none"> • Critical limit is WPS ≥ 6 percent. • Critical limits for killing parasites. |
| Addition of acids and/or preservatives: | <ul style="list-style-type: none"> • Critical pH limit ≤ 5. |
| Freezing step: | <ul style="list-style-type: none"> • Killing of parasites. |

Monitoring procedures, corrective action programme and verification procedures must be set up and records kept of all actions.

6.4.9. Mildly heat-processed fish products

A number of fish products receive a heat treatment during processing. Examples are: pasteurized or cooked and breaded fish fillets, cooked shrimp and crabmeat, cook-chill products and hot-smoked fish. After the heat treatment, the various products may pass through further processing steps before being packed and stored/distributed as chilled or frozen products. Some of these products may receive additional heat treatment before consumption (cooked and breaded fillets, cook-chill products) or they may be eaten without further treatment (hot-smoked fish, cooked shrimp). Thus, some of these products are RTE and extremely sensitive to contamination after heat treatment.

To further illustrate the safety aspects, there is ample epidemiological evidence that this type of product has been the cause of food poisoning owing to the growth of coagulase-positive *Staphylococcus aureus* and enteropathogenic organisms among the Enterobacteriaceae and Vibrionaceae. Marine crustaceans, usually shrimp, lobster or dishes made from them, accounted for 56 outbreaks involving 674 illnesses in the United States of America in the period 1998–2007 (CSPI, 2009).

In the application of the HACCP system to these types of products, the heat treatment is a very critical processing step. Hazards identified before this step may or

may not be eliminated depending on the degree of heat being applied. Most criteria for heat treatments have been laid down as a consequence of economic and technological considerations and not for hygienic or public health reasons. Increased safety will be obtained if the cooking/heating procedures could be designed to eliminate vegetative cells of pathogens and spores of the most sensitive species. Generally, a reduction of six orders of magnitude (six logarithms) in the level of contamination is recommended. This performance criterion is the so-called 6D process (“D” stands for “decimal reduction” – see also Section 2.2.1 for a discussion on “D”).

Listeria monocytogenes is normally used as a target organism for measuring the heat treatment and is regarded as the most heat-resistant food-borne pathogen that does not form spores.

Most products in this group depend entirely on the heating process and chilled storage for safety and shelf-life as they do not contain any bacteria-controlling ingredients. It is very likely that pathogens will cause disease if these factors are out of control. Pathogen survival during the cooking/heating procedure and pathogen growth during storage are significant hazards that must be included in the HACCP plan. In contrast, it is very unlikely that viruses, parasites and HPB will survive the heat treatment.

Recontamination of products after the heat treatment and before packaging can also cause consumer illness. In many cases, this hazard will be controlled by the prerequisite programme. In others, where, for example, the recontamination is caused by faulty container sealing or incorrect hot-filling procedures, recontamination is a significant hazard that needs to be included in the HACCP plan.

The possible presence of biotoxins and chemical contamination should be considered. Table 71 summarizes the hazard analysis of these products.

TABLE 71
Hazard analysis of mildly heat-processed fish

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Survival or re-contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	+	+	high	high	+	–	+	+
non-indigenous	+	+	high	high	+	–	+	+
Viruses	+	–	high	high	+	–	+	+
Biotoxins	+	–	high	high/low ²	+/-	+	–	+
Biogenic amines	–	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	low	–	–	–	–
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

In a simple production process (e.g. cooked shrimp vacuum-packed in plastic bags), the significant hazards are:

- survival of pathogens;
- recontamination after cooking;
- growth of pathogens;
- raw material quality (chemical hazards).

The CCPs during production will be:

- Receiving step: • Control of raw materials.
- Cooking step: • Control of survival of pathogens.

Recontamination and growth of pathogens will be taken care of by the prerequisite programme. The critical limits for the cooking step (time/temperature conditions) should be set at a point such that, if they are not met, the safety of the product may be questionable. If a more restrictive limit is set, the result will be a loss of product.

6.4.10 Heat-sterilized fish products packed in sealed containers (canned fish)

The basis for canning is the use of thermal processing to achieve commercial sterility of the final product. The containers are distributed at ambient temperatures and often stored for months, even years, under these conditions. The contents of the cans are normally eaten without any heating before consumption.

Canned fish has been the cause of outbreaks of botulism and cases of histamine and staphylococcal enterotoxin poisoning (Ababouch, 2002). The general hazard analysis is shown in Table 72.

Table 72

Hazard analysis of heat sterilized products packed in sealed containers (canned fish)

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Survival and/or re-contamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	+	+	high	high	+	–	+	+
non-indigenous	+	+	high	high	+	–	+	+
Viruses	+	–	high	low	–			
Biotoxins	+	–	high	high/low ²	+/-	+	–	+
Biogenic amines	+	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	low	–			
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

The significant hazards related to this type of products are:

- quality of raw material (biotoxins, chemicals);
- survival of pathogens (*C. botulinum*) during heat processing;
- presence of heat-stable toxins (biotoxins, histamine, *S. aureus* toxin);
- recontamination of product after heat processing (faulty containers, poor sealing, contaminated cooling water, faulty container handling).

The CCPs for these hazards are:

- Receiving step: • Hazard is the raw material quality.
- Quality of cans.
 - Critical limit: Cans must meet container specifications for safety.
 - Monitoring: Letter of guarantee from supplier. Visual examination of all lots of empty cans.
 - Corrective action: Reject defective cans. Contact supplier.

- | | |
|------------------------|---|
| Filling: | <ul style="list-style-type: none">• Corrective filling is important for proper heat penetration.• Visual check regularly (every half hour) by floor supervisor. |
| Sealing: | <ul style="list-style-type: none">• Faulty sealing may result in recontamination.• Can closures must be checked at regular intervals (every half hour) visually, and always when setting up a new machine or adjusting an old one. Tear down measurements must be done at the beginning of the shift and every 2 h thereafter by QC.• Corrective actions: Shut down processing line and inform plant manager. All products produced since last good check are put on hold. The cause of the problem must be identified before starting up again.• Any actions and measurements are recorded. |
| Retorting: | <ul style="list-style-type: none">• The hazard is survival of pathogens.• Critical limit is the botulinum cook or 12-D process.• If time/temperature requirements are violated, products must be put on hold for reprocessing and the cause must be identified. Records on all actions and measurements must be kept.• The verification programme should include a review of all operations and monitoring procedures and calibration of thermometers and automatic recorders. |
| Cooling: | <ul style="list-style-type: none">• Recontamination is possible if minute quantities of water enter the can. Use of chlorinated cooling water is a safe precaution. There must be measurable residual chlorine in the water (critical limit) and samples should be tested at least two times per day by a designated person (monitoring). |
| Post-process handling: | <ul style="list-style-type: none">• Contamination of hot and wet cans with <i>S. aureus</i> is prevented by isolation of the storage area of hot and wet cans and application of GHPs by personnel. |

Additional verification procedures are common practice and in some cases a legal requirement (EC, 1991). This includes checks carried out at random to ensure that products have undergone appropriate heat treatment. This requirement involves taking samples of the final product for:

- Incubation tests. Incubation of samples must be carried out at 37 °C for seven days or at 35 °C for ten days or any other equivalent combination.
- Microbiological examination of contents of containers in the establishment's laboratory or in any other approved laboratory.

6.4.11 Dried, smoke-dried, heavily salted fish

These are products with a very high salt content (> 10 percent WPS) and/or a very low water activity ($a_w < 0.85$). Dried or salted fish are usually considered stable at high temperatures and, therefore, stored and distributed at ambient temperatures.

No growth of pathogens is possible in these products if they are correctly processed, not even at ambient temperatures. The most salt-tolerant pathogenic organism is *Staphylococcus aureus* (which can grow at $a_w > 0.83$ and produce toxin at $a_w > 0.85$), and this organism should therefore be considered as a target pathogen for drying.

A critical phase in processing is the time until salt has penetrated and the WPS reaches 10 percent or the a_w is below 0.85 in the thickest part of the fish. For this reason, larger fish (> 15 cm in length) should be eviscerated prior to processing.

Contamination of dried or salted fish with enteropathogenic bacteria and viruses is a potential hazard, which will be prevented by the prerequisite programme.

The presence of toxic fish and chemical contamination of raw material are potential hazards.

The possible presence of parasites is not a significant hazard in these products. It is very unlikely they will cause a disease owing to the rapid killing of the parasites in an environment with a very high salt content.

When scombroid fish are used as raw material, the formation of histamine is a significant hazard. Histamine may be formed in the raw material before processing but also in the final product, as some halophilic bacteria are able to produce this compound (Kimm, Konagaya and Fujii, 2001). However, there is some uncertainty if this is a theoretical risk only. There are no reported cases of histamine poisoning from these products and there are no experimental data to demonstrate the possible risk. The general hazard analysis is shown in Table 73.

TABLE 73
Hazard analysis of dried, smoke-dried or heavily salted fish

Organism/ component of concern	Potential hazard		Analysis of hazard			Control		
	Recontamination	Growth	Severity	Likely occurrence	Significant	Government monitoring programme	PP ¹	Incl. in HACCP plan
Pathogenic bacteria								
indigenous	–	–						
non-indigenous	+	–	high	high	+	–	+	–
Viruses	+	–	high	high	+	–	+	–
Biotoxins	+	–	high	high/low ²	+/-	+	–	+
Biogenic amines	–	+	low	high/low ²	+/-	–	–	+
Parasites	+	–	low	low	–			
Chemicals	+	–	medium	high/low ²	+/-	+	–	+

¹ PP = prerequisite programme.

² Depending on fish/bivalve shellfish species, geographical position and season, the likely occurrence may be high or low.

The CCPs in the production of dried or salted fish are:

- Receiving step:
 - Hazard to be controlled is the raw material quality (presence of biotoxin, chemical contamination and histamine).
- Salting/drying step:
 - The hazard is growth of pathogens.
 - Critical limit is time to reach 10 percent WPS or a_w 0.85 in fish flesh.

6.4.12 Seafood risk categories

In ranking seafood into risk categories, the method of the NACMCF (1992), with some modifications, has been applied. The following six hazard characteristics and risk factors have been considered:

1. No terminal heat treatment. Apart from raw fish to be eaten cooked or fried, all other fish products are RTE.

2. The safety record. Is there any evidence that this particular product has been associated with food-borne disease many times – or with very serious diseases? It can be stated that the safety record is poor for:
 - a. molluscan shellfish and fish to be eaten raw owing to the presence of (accumulated) biological hazards (viruses, pathogenic bacteria, parasites and biotoxins);
 - b. molluscan shellfish, tropical reef fish and scombroid fish to be cooked before consumption owing to the presence of heat-stable aquatic toxins or scombrototoxin;
 - c. presence of heat-stable biogenic amines in canned sterilized products and few outbreaks of botulism caused by the same type of product;
 - d. some fermented fish, e.g. salted fish from the Near East or products from Alaska, the United States of America.
3. The production/processing does not include a CCP for at least one identified hazard. This situation applies to the:
 - e. accumulation of biological hazards in shellfish;
 - f. presence of biotoxins (ciguatera) in fish from tropical reefs.
4. The product is subject to potentially harmful contamination or recontamination after processing and before packaging. All raw fish and fish products that have not been subject to any bactericidal treatment are likely to harbour pathogenic organisms as part of their natural flora. Potentially harmful recontamination is possible and reasonably likely to occur for products being mildly heat-treated before being placed in the final container (cooked shrimp, hot-smoked fish). However, also the risk associated with lightly preserved fish and fish and shellfish to be eaten raw may increase due to this factor (e.g. contamination of cold-smoked fish with *L. monocytogenes*).
5. Products with a potential for abusive handling. This hazard refers mainly to handling and storing the fish product at abuse (elevated) temperatures. With the exception of sterilized, canned or fully preserved products, there is a potential for this hazard for all other types of fish products. However, this is not likely to occur for fish to be consumed raw, as spoilage will be very fast at elevated temperatures.
6. Growth of pathogens. The growth of pathogens, particularly in RTE products is a serious hazard. Two potential hazards of this nature are known and likely to occur: the possible growth of *L. monocytogenes* in lightly preserved fish products; and the growth of *C. botulinum* in some types of fermented seafoods. Growth of other pathogens in preserved or heat-processed products is possible only if the preserving parameters are not applied as specified (see text) and other potential hazards are in fact occurring (temperature abuse, recontamination of heat processed fish). Spoilage bacteria will grow in all types of fish products (except sterilized products) and, in most cases, they will grow faster than any pathogen. This is particularly the case in raw, unprocessed or unpreserved fish, and for this reason, growth of pathogens it is not considered an additional hazard likely to occur and influence the safety of this product.

The above considerations are summarized in Tables 74 and 75. The various seafoods are assigned to a risk category in terms of health hazards by using a “+” (plus sign) to indicate a potential risk related to the hazard characteristics. The number of plusses will then determinate the risk category of the seafood concerned.

TABLE 74

Risk categories for fresh seafood products

Seafood product	Characteristic that increases risk			Events that are reasonably likely to occur and that will increase risk			Risk category
	No terminal heat application	Bad safety record	No CCP for identified hazard	Harmful re-contamination	Abusive handling	Growth or accumulation of hazard	
Molluscan shellfish							
Live, raw	+	+	+	+	+	+	High ¹
Cooked	–	+	+	–	–	+	Medium
Raw fresh / frozen fish and crustacean							
Tropical reef	+	+	+	+	–	+	High
Scombroid	+	+	–	+	–	–	Medium
Other	+	–	–	+	–	–	Low
Fresh / frozen fish and crustacean to be cooked							
Tropical reef	–	+	+	–	–	+	Medium
Scombroid	–	+	–	–	+	+	Medium
Other	–	–	–	–	–	–	Low

¹ High-risk products have four or more plusses. Medium-risk products have three plusses. Low-risk products have two or fewer plusses.

Source: Modified after Huss, Jørgensen and Fønnesbech Vogel (2000).

Table 75

Risk categories for processed seafood products

Seafood product	Characteristic that increases risk			Events that are reasonably likely to occur and that will increase risk			Risk category
	No terminal heat application	Bad safety record	No CCP for identified hazard	Harmful re-contamination	Abusive handling	Growth or accumulation of hazard	
Lightly preserved (NaCl < 6%, pH > 5.0; e.g. cold-smoked)	+	–	(–)	+	+	+	High ¹
Fermented (NaCl < 8%, pH changing)	+	+	(+)	+	–	+	High
Semi preserved (NaCl > 6%, pH < 5.0; e.g. marinated)	+	–	–	–	+	+	Medium
Heat processed (hot-smoked, pasteurized)	+	–	–	+	+	+	High
Heat processed (canned, sterilized)	+	+	–	–	–	–	Low
Dried, smoke salted	+/-	–	–	–	–	–	Low

¹ High-risk products have four or more plusses. Medium-risk products have three plusses. Low-risk products have two or fewer plusses.

Source: Modified after Huss, Jørgensen and Fønnesbech Vogel (2000).

6.4.13 Example of development of an HACCP plan for the canning industry

Although generic HACCP plans can be found in many reports of training sessions and other proceedings of conferences and workshops that have been devoted to the subject, very few peer-reviewed publications have provided concrete examples of how an effective HACCP plan and its various components are developed. Instead, most address extensively the background and the principles of HACCP, including the food science and microbiology information necessary for HACCP plan development. This

is understandable because each HACCP plan should be tailored to the food operation considered, including the technical knowledge and experience of its team.

The following is a practical example to illustrate the development of an HACCP plan for canned sardines and mackerel. Section 6.10.4 describes another example in aquaculture – shrimp farming. It is important to stress that these examples are provided for illustrative purposes only – to demonstrate how hazard analysis is performed and how the Codex decision tree can be used to determine CCPs. They should not be adopted under any circumstances for similar seafood operations without adaptation and validation by a HACCP team.

6.4.13.1 Introduction

Company XYZ is specialized in the production of canned sardines and mackerel to be sold on the international market, mainly the markets of the European Union (Member Organization) and the United States of America.

Company XYZ has developed its HACCP plan in accordance with relevant provisions:

- The Codex Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003, Rev 2008).
- The requirements in the European Union Regulation 852/2004 on the hygiene of foodstuffs, Regulation 853/2004 laying down specific hygiene rules for food of animal origin (EC, 2004a), and Directive 2002/99/EC laying down health rules governing the production, processing, distribution and importation of products of animal origin (EC, 2002a).
- The requirements of the Food and Drug Administration (FDA, 1995): Title 21 of the Code of Federal Regulations Parts 123 and 1240, entitled “Procedures for the safe and sanitary processing and importing of fish and fishery products; Final Rule”. Federal Register, Volume 60 (No. 242, pages 65095-65202).

Company XYZ has adopted SSOPs as per the regulatory requirements for export to international markets. Consequently, the following HACCP plan development will address only process CCPs.

6.4.13.2 HACCP team

The HACCP team of company XYZ comprises:

- quality control (QC) manager (ABC);
- production manager (DEF);
- hygiene and personnel supervisor (GHI);
- maintenance supervisor (MNO);
- general manager (PQR);
- technical adviser (STU).

This team has expertise in food canning technology, food safety and quality and management. The team has developed formal communication channels with food control authorities, extension services, public health authorities and clients to ensure appropriate development of the HACCP manual. Table 76 provides the necessary information on the HACCP team, its qualifications and duties.

Each team member is responsible for carrying out the duties identified for him/her in the plan, under the supervision of the QC manager, who validates all actions necessary for the implementation of the HACCP plan. If needed, the QC manager will refer to the general manager for the implementation of cumbersome and costly actions, presenting the different options and solutions without any compromise on safety and quality, as per company policy. If necessary, the technical adviser is consulted to provide scientific and technical advice as seen fit.

TABLE 76

HACCP team of company XYZ

Name	Background and experience	Title/responsibility	Duties
ABC	Food science degree Certificate of HACCP course from by the University of... FDA Better Process Control School BPCS certificate from the University of ... 10 years experience in food and fish safety and quality	QC manager responsible for the implementation and revision/maintenance of the company's HACCP manual	Supervises the elaboration of the HACCP manual Supervises and coordinates the implementation of QC activities (sampling, analyses, supervision of corrective actions) In charge of the calibration and validation of control methods Supervises training of company personnel in QC matters Handles complaints of clients and food control agencies and follows up on corrective action to these complaints Updates list of suppliers of fish, salt, oil, packaging materials Maintains the HACCP plan and its revisions
DEF	Food science degree GMP certificate from the University of.... HACCP certificate from the veterinary school ... 7 years experience in food processing	Production manager responsible for the daily running and planning of the production through storage and shipment	Participates in the elaboration of the HACCP manual Plans and supervises production Supervises training of company staff on technology issues Implements control measures and corrective actions under the guidance of the QC manager Revises the list of suppliers of inputs, in collaboration with the QC manager Assists in the revision of HACCP plans
GHI	Food science certificate from the University of... Certificate of GHP from vocational training centre 3 years experience in food hygiene	Hygiene and personnel supervisor in charge of implementing GHPs and cleaning and disinfection programmes	Participate in the elaboration of the HACCP manual Supervises training on GHPs Develops and revises cleaning and sanitation programmes Plans and coordinates pest control programmes
JKL	Food technology certificate from the University of... Maintenance certificate from vocational training centre... 5 years experience in maintenance	Maintenance supervisor in charge equipment maintenance, in particular, retorts and seaming machines	Plans and coordinates plant and equipment maintenance operations; Plans and coordinates plant and equipment maintenance operations subcontracted to outside companies
MNO	No formal advanced qualification, but practical experience at all levels of the business	General manager in charge of managing the logistics and administration of the company	Draws up the quality and safety policy of the company Approves the HACCP plan and its revisions Commits the resources to implement HACCP Chairs monthly meetings of the HACCP team to review progress and address issues. Minutes of the meetings are recorded, filed and distributed to HACCP team
PQR	Degree in food safety and quality from ... 15 years experience in fish and seafood safety and quality	Technical adviser	Supervises the elaboration of HACCP manual and its revision Carries out the yearly audit of the HACCP system Provides advice and relevant information on emerging issues, regulations, safety and quality management guidance

6.4.13.3 Product description

Company XYZ manufactures 25 different canned seafood products. Examples of these products are described in Table 77.

TABLE 77
Example product descriptions for canned products

Product	Contents	Packing materials and format	Shelf-life
Canned sardines in vegetable oil	Beheaded tail-off sardines: 75% Soya oil: 24% Salt: 1% Water activity $a_w = 0.98$ pH = 6.2 – 6.5.	(1) Tin format 1/6 P 30, 2 pieces, simple or easy open lid (2) Aluminium alloy format 1/6 P 30, easy open lid (3) ½ H 40 (4) 1/6 P 30 DAS R 26	5 years at ambient temperature
Canned sardines “au naturel”	Beheaded and tail-off sardines: 75% Water: 24% Salt: 1% Water activity $a_w = 0.99$ pH = 6.2 – 6.5.	Tin or aluminium alloy (1) Format 1/6 P 30, 2 pieces, simple lid (2) 1/6 P 30 ES, easy open lid All cans are individually packed in paper holsters	3 years at ambient temperature
Skinless, boneless canned sardines in olive oil	Skinless/boneless sardine fillets: 75% Olive oil: 24% Salt: 1% Water activity $a_w = 0.98$ pH = 6.2–6.5.	Tin or aluminium alloy (1) format 1/6 P 30 2 pieces, simple lid (2) 1/6 P 30 2 pieces, easy open lid (3) 1/6 P 22 2 pieces, easy open lid All cans are individually packed in paper holsters	5 years at ambient temperature
Mackerel filets canned in tomato sauce	Mackerel fillets: 75% Tomato paste: 22% Soya oil: 2% Salt: 1% Water activity $a_w = 0.98$ pH = 5.8–6.1.	Tin or aluminium alloy (1) format 1/6 P 30, 2 pieces, easy open lid All cans are individually packed in paper holsters	3 years at ambient temperature

6.4.13.4 Intended use of the product

The canned fish produced by company XYZ is destined for export mainly to Europe and the United States of America. It is generally consumed without any cooking, as an appetizer, in a sandwich or after mixing with other food or salads. It is consumed by the public at large, with no specific age restriction.

6.4.13.5 Construction of the flow diagram

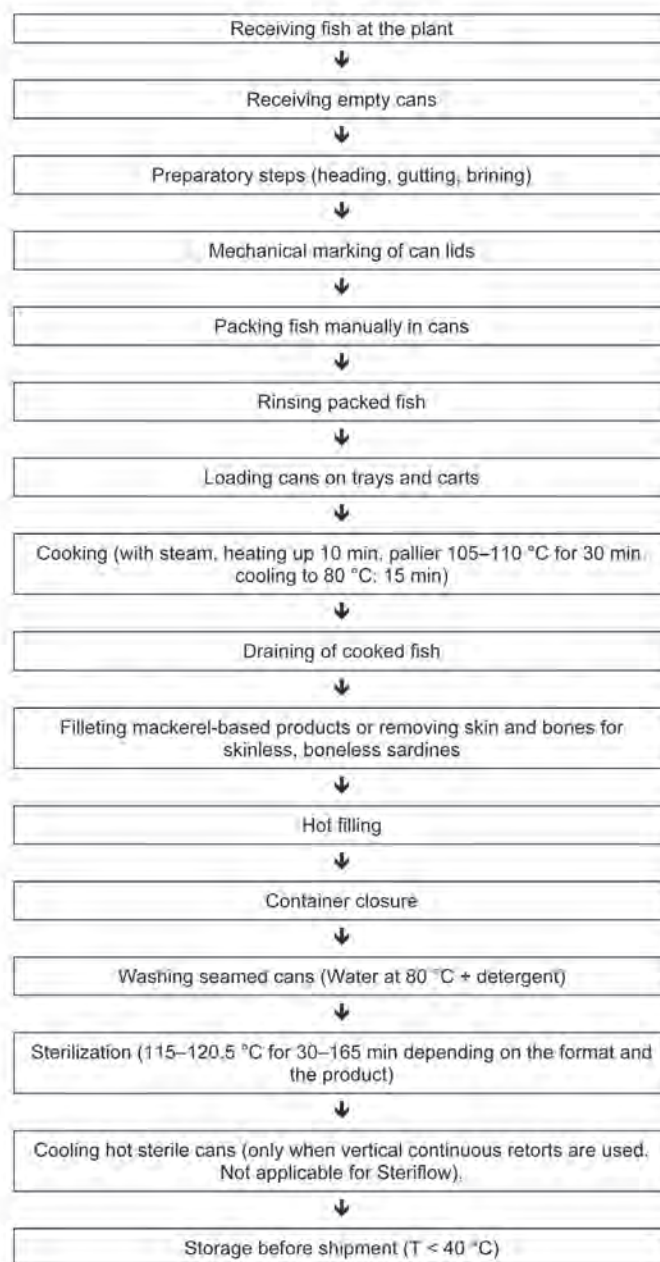
On DD/MM/YYYY, the HACCP team reviewed the current operations used for the production of canned fish at company XYZ, collected the necessary information and constructed the flow diagram presented in Figure 45.

The thermal process has been certified by a thermal process authority. It operates at $F_0 = 7$ –14 minutes, depending on the product and format. The thermal process was registered with the FDA on DD/MM/YYYY. The following details were provided for both certification and registration: type of retort, minimal F_0 , can format, sterilization temperature, heating duration, minimal initial temperature, filling method, ratio solid/liquid, stacking of the cans in the retort basket, number of baskets per retort, sterilization system (steam heating, venting, initial temperature, start time).

6.4.13.6 On-site confirmation of flow diagram

On DD/MM/YYYY, the HACCP team verified carefully the different steps of the flow diagram, synthesized them into one diagram that was complemented with data and information relevant to HACCP, such as fish temperature, brine strength, sterilization temperatures and times, product pH and water activity, can formats, and flow rates. The verification was carried out in the cannery, which was operating at full capacity. All the collected data were recorded, consolidated and used to update the flow diagram (Figure 45).

FIGURE 45
Flow diagram of canned sardines and mackerel at company XYZ



6.4.13.7 Hazard analysis

Potential hazards that can compromise safety and quality have been studied and analysed by the HACCP team. To do so, the HACCP team relied on the expertise of its members, the feedback of its clients and that of the food control services, the technical specifications of its clients, and other information available with public health authorities, extension services and on authoritative technical and scientific publications.

The potential hazards identified were either contamination (from fish, water, ice, equipment or personnel) or survival (after sanitation, cooking and sterilization) of hazardous micro-organisms, the production or persistence of toxic chemicals (such as histamine, staphylococcal enterotoxins and botulinum toxins). For each hazard, the most appropriate preventive measure was identified.

Concerning the persistence of heavy metals, especially mercury, in canned fish, it was not considered a real hazard because data on finished products, available to company XYZ for more than 10 years, indicate levels far below 0.5 ppm in raw material. However, company XYZ exercises care in this regard before processing fish caught in areas different from the traditional ones or in case of any alert given by the food control agency, which carries out a monthly surveillance programme of heavy metals in fishing grounds.

Although different hazards present varying levels of severity and likelihood of occurrence, the HACCP team considered all identified hazards and quality defects important and identified control measures to eliminate each hazard or reduce it to acceptable levels, to meet regulatory requirements of importing markets and to avoid rejections or detentions of shipments at international borders or by buyers.

The details of hazard analysis are presented in Appendix 2. The results of the hazard analysis, including control measures, are summarized in Table 78.

TABLE 78
Hazard analysis of company XYZ

Hazard	Severity	Risk	Control measures
Botulism because of insufficient thermal processing or because of post-process contamination during cooling	+++++	+	Proper sterilization Proper training of personnel in charge of sterilization Proper chlorinating of cooling water
Histamine poisoning because of contaminated raw fish or histamine accumulation during preparation.	++	+++	Training of purchase supervisor in proper freshness assessment Proper icing and refrigeration Control of histamine level at receiving when in doubt.
High levels of heavy metals	++++	+	Good knowledge of fishing zones Ensuring the purchase of fish caught only in pollution-free areas
Post-process contamination with pathogens or toxic materials because of bad container closure	+++++	+	Training of container closure supervisor Maintenance of seaming equipment
Staphylococcal poisoning because of bad handling of wet and hot freshly sterilized cans	+++	+	Air drying of wet cans Storage of wet cans in restricted-access area

6.4.13.8 Identification of critical control points

Once the hazard analysis had been carried out, the step where each hazard might appear in the flow diagram and its cause (or causes) were identified by the HACCP team. Then, each of the flow diagram steps was assessed to determine whether it was a CCP or not. To do this, the HACCP team relied on its expertise and used the decision tree recommended by Codex (Figure 42). The details of CCP identification are presented in Appendix 2.

6.4.13.9 Establishment of critical limits

For each identified CCP, the HACCP team designed a critical limit to assess whether the preventive/control measure was applied correctly to eliminate the hazard or reduce it to an acceptable level. Again, the HACCP team relied on its expertise, regulatory limits, guidance and specifications from clients. As much as possible, critical limits were set as operational limits, i.e. limits that indicate a slide towards a loss of control but before the manifestation of the hazard. All the critical limits are presented in Appendix 2.

6.4.13.10 Development of a monitoring system

The HACCP team developed a monitoring system to check conformity to the targeted critical limits, based on its expertise, experience and advice compiled from relevant documents, regulations and clients' specifications. The monitoring procedures, including sampling plans where relevant, are described in Appendix 2 (Annex A2.II).

6.4.13.11 Identification of corrective actions

Again, relying on its experience and expertise and relevant documents, regulations and clients' specifications, the HACCP team identified corrective actions to be activated when monitoring indicated loss of control, as well as the respective communication and command chains to implement the corrective actions. These actions and the procedure to implement them are presented in Appendix 2.

6.4.13.12 Verification procedure

On a monthly basis, the QC manager assesses internally all the results of the controls, monitoring and corrective actions and draws conclusions for the following production cycle. The QC manager prepares a report for the meeting of HACCP team, chaired by the general manager. Recommendations from the meeting are implemented by the staff concerned under the supervision of the QC manager.

For the longer time frame, company XYZ has set up an annual verification procedure that comprises:

- Evaluation of all the inspection data obtained from the laboratory of the food control authority. This laboratory carries out chemical analyses (total volatile bases [TVB] and histamine, commercial sterility and mercury) on each lot of finished product before shipment. All these data are analysed to assess the quality level of the production over the year. Any quality problem detected by these analyses will be immediately addressed by the QC manager to identify why the HACCP system did not operate properly to prevent the problem.
- Evaluation of the feedback information from the clients.
- Evaluation of the monitoring and corrective actions data to assess performance and analyse the reason for any loss of control or for any complaint from clients and/or the food control authority.
- The results of this analysis are used to update the HACCP manual, identify any internal need for further training and improved practices, performance and maintenance, modify frequency (increase or decrease) of specific monitoring, and revise list of approved suppliers to eliminate unreliable ones.
- An audit by the technical adviser to assess the performance of each control, monitoring or corrective procedure. The adviser audits the different records, including records for monitoring, calibration and maintenance, training, complaints and reports from clients and control authorities. The adviser prepares a report that is submitted to management and discussed during a meeting with management and the HACCP team. The audit exercise is also used as an opportunity to introduce new procedures, monitoring techniques or critical limits in order to take into consideration new developments, including new regulatory requirements.

6.4.13.13 Record-keeping procedures

Forms are used to record the results of each monitoring activity and any corrective action that is implemented. These forms identify who is responsible for the implementation of preventive (control) measures, monitoring and corrective actions, and who should validate these actions or be informed of their respective outcome as per the duties described in Table 76. Example forms can be found in Appendix 2.

6.5 APPLICATION OF THE HACCP PRINCIPLES FOR THE MANAGEMENT OF FISH QUALITY (LAHSEN ABABOUCHE)

While the HACCP principles and concepts of farm-to-fork have been developed to ensure food safety, the approach and thinking underlying them can readily be applied to cover other quality aspects, such as sensory quality, composition or labelling. Instead of identifying the hazards of the process or product, potential defects are considered. The steps or points at which the defects are to be controlled are called defect action points (DAPs) (CAC, 2003) as a parallel to the CCPs, where hazards can be controlled. Similar to the CCP procedures, the limits, monitoring procedures, corrective actions and verification procedures must be established at the DAPs.

Defect	Defect Action Point (DAP)
A condition found in a product that fails to meet essential quality, composition and/or labelling provisions of the appropriate Codex product standards. (CAC, 2003)	A step at which control can be applied and a quality (non-safety) defect can be prevented, eliminated or reduced to an acceptable level, or a fraud risk eliminated. (CAC, 2003)

The analysis of potential defects and identification of DAPs follows the same procedures as when conducting a hazard analysis. For example, the decision tree used to determine whether a point is really a CCP can be used equally well to decide whether a given point is a DAP.

Defects may, as hazards, be of a biological (microbiological), chemical or physical nature. The substitution of one (lower-value) fish species for another (high-value) one is an example of a biological defect. Whether intentional or not, it is fraud. Similarly, raw materials for production of semi-preserved herring must have a specific lipid content for the right ripening and texture to develop. Therefore, lower or higher lipid content is a biological defect. This should be monitored on the incoming raw material, and batches with unsuitable lipid content should be used for other products.

Other kinds of defects include incorrect weight or incorrect labelling.

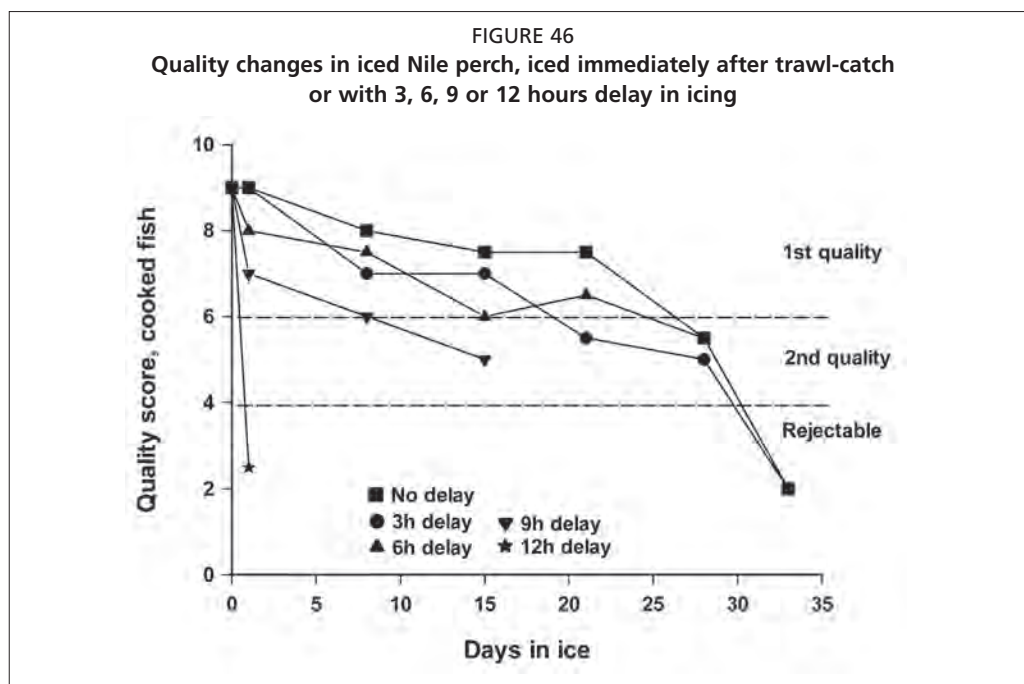
6.5.1 Microbiological aspects

This technical paper has so far focused on the risk to consumer health arising from the presence and growth of micro-organisms. However, micro-organisms may have other adverse effects on the quality of fish and fish products. Thus, growth and activity of micro-organisms is the major cause of decomposition (spoilage) of all types of products where micro-organisms have not been completely inactivated (such as in canned foods) or where the growth of micro-organisms has not been completely arrested (such as in frozen foods). A description of the spoilage patterns of different fish products and the micro-organisms involved can be found in Huss (1995), Gram and Huss (2000) and Gram *et al.* (2002).

It has been estimated that between 10 and 50 percent of all foods produced are lost post-harvest or post-slaughter owing to microbial activity (Kaferstein and Moy, 1993; Baird-Parker, 2000; WHO, 1995). Decomposition or presence of filth is the most common cause of detention of fish products imported into the United States of America – out of 1 858 import refusals related to fish and fishery products in the in 2010, 706 were due to filth (NFI, 2011). Organoleptic causes accounted for the 56 of

the 703 rapid alerts associated with fish and fishery products in 2011 in the European Union (Member Organization).¹³

In principle, control of decomposition of fish and fish products is simple as low temperature will retard all spoilage processes. In contrast, just a few hours exposure to high temperatures may accelerate spoilage. In several tropical countries, icing is not done on board the fishing boats, especially for low-value species, and this leads to rapid reduction in eating quality (Figure 46). It also follows indirectly from the figure that temperature during storage is critical.



Source: Gram (1989).

Loss of quality occurs rapidly. Therefore, control of the time and temperature is critical. This DAP applies to all steps from catch, through processing and distribution to the consumer. More recent innovations allow monitoring of the accumulated time-temperature using small data loggers. However, the most efficient and reliable way of determining whether or not this DAP is under control is sensory evaluation.

Monitoring of time and temperature during handling and processing can be done by date-marking of boxes and containers and by visual inspection of icing and chilling conditions. Time and temperature recording at specific points and during processing should preferably be controlled automatically. Process flow must be designed to avoid stops and interruptions, and chill rooms must be supplied with thermometers. Visual inspection (e.g. quantity of ice) and control checks of temperature must be done in a daily routine. A log of temperature recordings (done manually or automatically) must be kept and be available at all times.

Off-flavour may also arise in fish owing to microbial growth that is not related to spoilage aspects. The muddy flavour often detected in freshwater fish such as trout is caused by the compound geosmin. Blue-green algae, actinomycetes and cyanobacteria are capable of producing geosmin. The compound accumulates in the fish flesh and is not toxic to fish or humans. Again, sensory evaluation is the most reliable detection

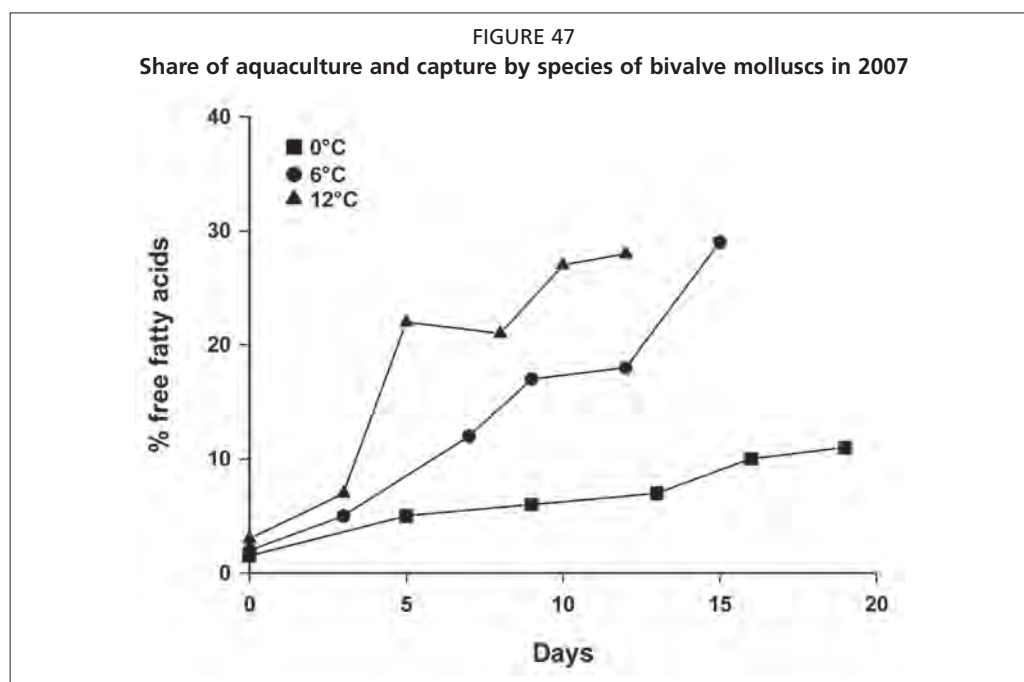
¹³ Data from the EU RASFF portal: <https://webgate.ec.europa.eu/rasff-window/portal/>

technique. Allowing the fish to swim in clean water for 4–7 days before harvesting can reduce (purge) the off-flavour.

6.5.2 Chemical aspects

As presented in Chapter 4, chemical defects refer to quality deterioration due to chemical reactions. Very common are the changes that may occur in the fish lipid fraction through either oxidation or hydrolysis. Both reactions result in the production of substances with unpleasant – rancid – off-flavours. Other changes such as dehydration and autolysis may lead to poor texture and freeze burns. During frozen storage, especially of gadoid fish species, trimethylamine oxide (TMAO) is reduced to dimethylamine (DMA) and formaldehyde (FA). This adds to the changes in texture and flavour occurring during frozen storage.

Availability of oxygen (or other oxidizing compounds) is required for oxidative rancidity to develop, and non-oxygen-containing packaging of fatty-fish species will control this defect. As with microbial reactions, temperature is important. Thus, the development of free fatty acids in herring is greatly accelerated at 12 °C as compared with 0 °C (Figure 47).



Source: Redrawn from Huss (1995).

Any contamination occurring during processing that is not included as a hazard in the HACCP plan will also constitute a defect. This could be contamination (or recontamination) by cleaning agents, by mechanical grease or by using wrong ingredients.

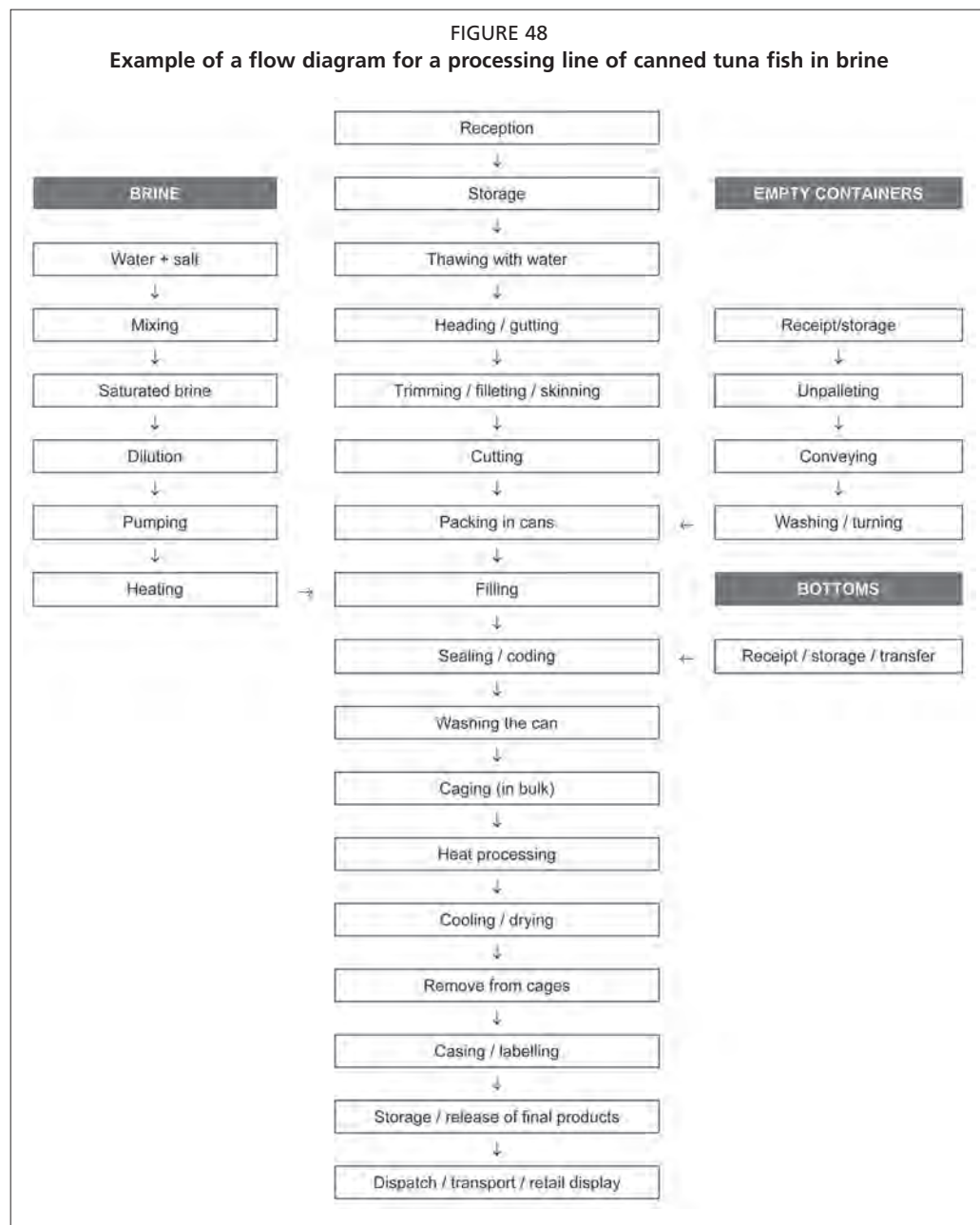
6.5.3 Physical aspects

Defects of a physical nature cover a range of aspects such as the presence of small bones, foreign matter (e.g. hairs or straw) or material that should not be there (scales, pieces of skin, etc.). Other physical defects can damage the packaging, causing bruising to the product or change of carton shape.

6.5.4 Example

The CAC (2003) provides a good example of the use of defect analysis and identification of DAPs (Tables 79–81). As with the hazard analysis, the production flow must first be outlined (Figure 48). The defect analysis identifies several possible defects (Table 79).

As outlined, spoilage is mainly a problem of the time and temperature control of the non-frozen or non-canned fish. Further analysis points to the development of rancid off-odours as a potential defect. Each processing step should then be considered to determine whether it is a possible action point for the defect. Table 80 illustrates the preliminary analysis of step two in the fish flow, i.e. the frozen storage step. As the frozen tunas are often stored in bulk, the frozen storage period could be a potential DAP.



Source: Adapted from CAC (2003).

TABLE 79

An example of potential defects of canned tuna

Defect type	In raw tuna	During processing, storage or transportation
Biological	Spoilage	Spoilage, survival and growth of spoilage micro-organisms
Chemical	Oxidation	Oxidation
Physical		Objectionable matter (viscera, scales, skin, etc.), formation of struvite crystals, container defects
Other	Species substitution	Abnormal flavours, incorrect weight, incorrect coding, incorrect labelling

Source: Modified from CAC (2003).

TABLE 80

An example of the significant defect rancidity during the storage of frozen tuna for canning tuna

Processing step	Potential defect	Is the potential defect significant	Justification	Control measures
Storage of frozen tuna	Persistent and distinct rancid odours and flavours	Yes	Product does not meet quality or customer requirements	Glazing Controlled temperature in the storage premises Packaging Stock management procedure Maintenance of procedure of the refrigeration system Personnel training and qualifications

Source: Modified from CAC (2003).

The analysis indicates that the frozen storage could be a DAP for development of rancid off-odours. A more detailed analysis – similar to the decision tree for CCPs – is presented in Table 81.

TABLE 81

A schematic example of a defect analysis with corresponding control measures and the application of the Codex decision tree for the determination of a defect action point during storage of frozen tuna

Q1: Do control measures exist?	Q2: Is the step specifically designed to eliminate or reduce the likely occurrence of rancidity to an acceptable level?	Q3: Could rancidity occur in excess of acceptable levels or could it increase to unacceptable levels	Q4: Will a subsequent step eliminate rancidity or reduce its likely occurrence to an acceptable level?
If yes – go to Q2		If yes – go to Q4	If yes – not a DAP
If no – consider whether control measures are available or necessary within the process	If yes – this step is a DAP If no – go to Q3	If no – not a DAP	If no – DAP
Proceed to next identified defect			
A: Yes, the storage temperature is controlled, procedures exist	A: No	A: Yes, if the storage time is too long and/or the storage temperature is too high or if packaging is broken or unsuitable, or if glazing is inadequate	A: No
Decision: Storage of frozen tuna is a defect action point			

Note: Q = question; A = answer.

Source: Modified from CAC (2003).

6.6 HACCP AUDITING AND VERIFICATION (LAHSEN ABABOUCHE)

Application of HACCP in the fish and aquaculture industry is the responsibility of the production and processing industry, whereas government control agencies are responsible for monitoring and assessing proper implementation of prerequisite programmes and HACCP.

Many inspection agencies have developed approaches and procedures for carrying out HACCP compliance auditing. These approaches and modalities have used the terminology and basic requirements of the ISO 10011 (and more recently ISO 19011-2011) standards that were adapted to the specificities of HACCP and to the countries' regulations. Information regarding these procedures is not reviewed here in detail as it is widely accessible, especially via the Internet. This section attempts to clarify the issues and advise on how to achieve practical HACCP auditing.

6.6.1 Planning and conducting an HACCP audit

An audit is a systematic and independent examination to determine whether activities and results comply with the documented procedures and also whether these procedures are implemented effectively and are suitable to achieve the objectives. In HACCP terms, achieving the objectives means managing the production and distribution of safe and good-quality fish products through the use of an HACCP-based approach.

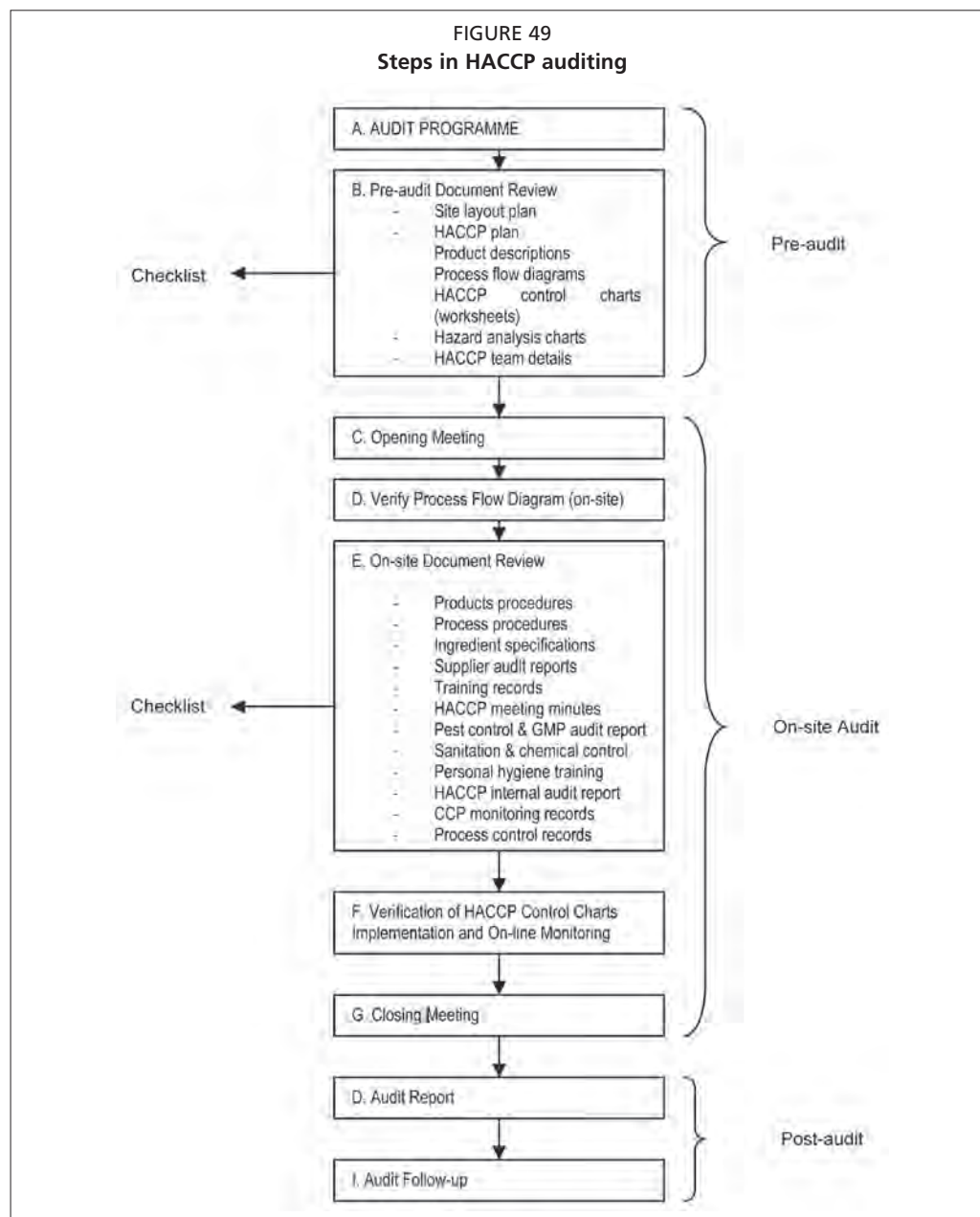
The outcome of the audit is to establish whether the manufacturer has:

- developed and implemented a sound HACCP system;
- the knowledge and experience needed to maintain it;
- the necessary support (or prerequisite) programmes in place to assess adherence to GHPs/GMPs.

The audit will encompass assessment of the management commitment to support the system and assessment of the knowledge, competence and decision-making capabilities of the HACCP team members to apply the system and maintain it. Four types of HACCP audits can be envisaged:

- An internal HACCP audit to establish the effectiveness of the HACCP system using the company's own human resources or by bringing in an external HACCP assessor.
- An external HACCP audit of suppliers of raw materials or finished products to establish whether they have robust HACCP systems in place. This includes regulatory HACCP auditing.
- Audit of the customer's HACCP system. This may be important where the customer is responsible for the distribution and sale of a high-risk (e.g. a chilled ready meal) product that bears the brand of the manufacturing company.
- An investigative audit can also be conducted to analyse a specific problem area. This may be used, for example, when a CCP regularly goes out of control and more studies are needed to investigate the real cause in order to take corrective action, or where a previously unknown problem has arisen.

An HACCP audit needs to be properly prepared. Figure 49 describes the steps generally required in an HACCP audit. This guidance is useful for independent (third-party) audits as well as for internal or compliance audits. It should be adapted to the particular circumstances of the firm being audited.



Source: Mortimore and Wallace (1998)..

6.6.1.1 Pre-audit

A preparatory phase is necessary to elaborate the schedule and the definition of the scope of the audit. All the personnel required during the audit should be notified to ensure that they are available. In addition, the necessary documentation should be made available for the audit.

This starts with a “desktop assessment” of the HACCP system to review all of the documentation relating to the scope of the audit, such as the flow diagram layout, the time/temperature and other technological information, the hazard analysis, etc.

The pre-audit document review can be done as an initial scan to form a picture of who carried out the HACCP study, its style, its completeness, and also familiarization with the site being audited and the products and process itself. It will give an opportunity for the auditor to carry out some research before the assessment. At this stage, it is important to build up knowledge of the product/process technology

concerned. Literature searches of the technology, fish contamination outbreaks and legislative controls should be included. Guides and other support documents can be useful.

It is also important to gauge the level of commitment of the management and the competence of the HACCP team members by assessing their training and experience.

If the pre-audit indicates obvious inadequacies, it may be advisable to stop the assessment at this point prior to the on-site audit. The deficiencies should be discussed with the HACCP team members, who can then review their HACCP system and implement any required corrective measures.

6.6.1.2 On-site audit

An opening meeting is useful to present the team of auditors, the scope and the tentative timetable and to identify the personnel and documentation required.

At this stage, the accuracy of the process flow diagram will be carefully checked, followed by a full review of operational procedures for CCP monitoring, CCP monitoring records, training records, etc. The prerequisite GMPs and hygiene maintenance records, pest control and also the HACCP team meeting minutes can be reviewed. In the latter case, it may be helpful to use this to form an idea of the decision-making process, who attended the meetings on each occasion, and whether difficulties were encountered. The review will also include previous audit records where non-compliances may have been found. The assurance of the effectiveness of any corrective actions taken must be sought. Other quality- and safety-related data for review will include customer complaints and customer audit reports.

It is often useful to use checklists during the audit. An example of a checklist is presented in Table 82. The “considerations” column can be completed during the document review step of the process, and the “auditors findings” column during the conduct of the audit itself.

During a closing meeting, the overall assessment findings are presented and an overall view of the proceedings is given. Non-compliances should be discussed together with supporting evidence and a schedule for the corrective actions agreed. The auditor must ensure that identified deficiencies are clearly understood and that the recommended corrective actions are feasible and agreed by a senior manager.

6.6.1.3 Post-audit

Audit reports should provide evidence of the findings of the assessment – primarily, what deficiencies have been found in the HACCP system, the non-compliance notes, the recommended corrective measures and the timetable to implement them.

During the audit follow-up, the auditor should ensure that the non-compliances are closed off. The effectiveness of corrected non-compliances should be verified as soon as the corrective action has been taken and reviewed during subsequent audits to ensure that the corrective actions taken have been effective on an ongoing basis

6.6.2 Frequency of audit

The frequency of HACCP audits should be based on:

- the risk category of the fish product being processed;
- the level of commitment of management and the decision-making leverage of the HACCP team;
- the reputation of the fish company: previous safety and quality records, HACCP manual and implementation classification, training and qualification.

TABLE 82
Example of a checklist for assessing HACCP implementation

Component to assess	Compliance, considerations, points to raise on-site	Findings of the auditor
(1) Commitment of the management		
Financial commitment		
Awareness/support		
(2) HACCP team		
The HACCP team leader has effective power of decision		
The HACCP team members are qualified		
(3) Composition of products		
Fish composition is properly described		
Any modification is recorded and taken into account for HACCP revision		
(4) Intended use		
Valid description of the intended use		
Any modification is recorded and taken into account for HACCP revision		
(5) Process flow diagram (or diagrams)		
The flow diagram is correct		
Any modification is recorded and taken into account for HACCP revision		
(6) Hazard analysis		
All control measures are correctly implemented, and validated as necessary		
Personnel in charge of control measures are identified and qualified		
New hazards, introduced because of changes in product or process, are taken into consideration		
Control measures have been identified for these new hazards		
(7) Critical control points (CCPs)		
CCPs are properly identified (e.g. using the decision tree)		
Introduction of new hazards has resulted in CCP analysis to implement proper control measures		
(8) Critical limits		
Critical limits are properly identified and validated as necessary		
Introduction of new hazard has resulted in the revision of the critical limits		
(9) Monitoring procedures		
Monitoring procedures are properly identified		
The reliability of the monitoring procedures has been validated		
Personnel in charge of monitoring are well identified and trained		
All necessary modifications have been made to take into account the introduction of new control measures		
(10) Corrective actions		
Corrective actions are properly identified and validated as necessary		
Personnel in charge of corrective actions has been identified and trained		
All necessary modifications have been made to take into account the introduction of new control measures		
(11) Verification of the HACCP system		
The method and frequency of verification are appropriate		
The validity of the verification method has been confirmed		
Personnel in charge of verification are identified		
Changes of products, processes, standards and regulations, etc. are taken into consideration		
(12) Record-keeping system		
Forms are appropriate and complete		
Forms are up to date for recording the following:		
• monitoring results		
• corrective actions		
• modifications of the HACCP system		
• HACCP verification/revision results		
Some records have been tampered with		

Source: Ababouch (2000).

6.6.3 HACCP approval/certification

An HACCP audit exercise should lead to an audit report that should state whether the system provides enough assurance to control fish safety and quality. However, fish processors look for a formal recognition (validation, certification). It should be stressed that although this is legitimate, an HACCP audit is a snapshot evaluation at a point in time, and any recognition should not lead to false assurance. It is a temporary recognition, and audits should be as frequent as seen fit.

In international fish trade, there is a danger of duplication of HACCP audit efforts. This can be alleviated by the development of an internationally recognized equivalence system, for example, through the Codex Committee on Food Import and Export Inspection and Certification Systems.

Furthermore, third-party certification can complement the work of government inspectors in assessing HACCP. However, certifying bodies should demonstrate proper qualifications and integrity in HACCP development and verification. This may require the establishment of a certification system for third-party HACCP assessors.

6.6.4 Qualifications of HACCP auditors

Proper assessment of HACCP requires demonstrated knowledge and qualifications in different areas of science and technology pertinent to the products and processes of interest, in addition to confidentiality, objectivity and experience and skills in auditing and communication. These qualifications are acquired through training and experience. It should be stressed that any training activity should provide evidence of satisfactory completion through examination. In addition, the training programmes and examinations should be harmonized to allow for easy recognition and equivalence between countries.

6.7 TRACEABILITY (MARCO FREDERIKSEN)

An important aspect of quality and safety assurance is to be able to trace products, ingredients, suppliers, retailers, processing operations or storage procedures through the food production chain. This is especially relevant when failures occur. The term “traceability” has been introduced to describe systems in which information about a particular attribute of a food product is systematically recorded from creation through to marketing (Golan, Krissoff and Kuchler, 2002). For example, if a particular batch of cold-smoked fish has caused an outbreak of listeriosis, authorities will want to trace the product in question to the producer and distributor to establish re-call procedures. Similarly, the producer will want to determine whether contamination with *L. monocytogenes* occurred in the plant and/or whether temperature abuse occurred during distribution or during storage at the retailer or at the consumer. One may regard an epidemiological investigation as part of a traceability study, e.g. determining the sources of an agent involved in an outbreak of food-borne disease.

Traceability systems have been used for many years in several other sectors such as the aviation, automobile and pharmaceutical industries. As the food chain has lengthened from local production, processing and consumption to more global commercial opportunities, the need to transfer information related to production and public health and the complexity of these transfer vehicles have expanded (McKean, 2001). With the increase in complexity, the consumer wishes to know the origin (species, place, condition of rearing or catch area), the transformations and the distribution of their food products (Pascal and Mahé, 2001).

Quantitative risk assessments typically aim at covering the whole “farm-to-fork” chain and, at any point in time, one must, therefore, be able to trace an event or a product.

Four international definitions on traceability are listed:

1. The Codex Alimentarius Commission (CAC, 2011) defines traceability as: The ability to follow the movement of a food through specified stage(s) of production, processing and distribution.
2. ISO 9000 (ISO, 2005) defines traceability as: The ability to trace the history, application or location of that which is under consideration. When considering a product, traceability can relate to:
 - a. the origin of materials and parts
 - b. the processing history
 - c. the distribution and location of the product after delivery.
3. ISO 22005 defines traceability as: The ability to follow the movement of a feed or food through specified stage(s) of production, processing and distribution. Movement can relate to the origin of the materials, processing history or distribution of the feed or food but should be confined to one step forward and one step backward in the chain.
4. The European Union (Member Organization) (EC, 2002a) defines traceability as: The ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution.

The definitions are very similar but the ISO 9000 definition covers all products in general whereas the three others only apply to food and feed.

In general, the term “trace” or “tracing” is used when the history of product origin is searched (upstream), and the term “track” or “tracking” is used for searching its history after delivery (downstream). Moe (1998) described the terms used in traceability studies in the following way:

- A step refers to a discrete operation or location at which some task or process is performed on the product.
- A chain is composed of the sequence of these steps.
- A product can be any material at any stage of processing, e.g. a live fish, a whole fish, or a processed fish product.

Withdrawal refers to the removal of goods before they are delivered to consumers, while recall refers to the removal or taking back of goods when the goods already are available at the retail level (FAO, 2006). Olsen and Borit (2013) have undertaken an in-depth review on the definition of traceability and offer a new definition as it relates to food products.

Interest in traceability in food processing has been increasing in recent years, primarily because of different scandals in the food sector such as mad cow disease (bovine spongiform encephalopathy – BSE) in 1996 in the United Kingdom of Great Britain and Northern Ireland, and dioxin contamination in Belgium in 1999. Authorities have focused on traceability to ensure consumer safety, to be able to recall defective/hazardous products and to identify the source of the problem.

The attack in the United States of America on 11 September 2001 increased the focus on traceability. Today, traceability is demanded from food producers by national legislation in both the European Union (Member Organization) and the United States of America. Traceability is also required by most supermarket chains, and their requirements are often higher than those established by national legislation. Traceability has thus become a requirement for market access.

Traceability may also be advantageous within a company by allowing different raw materials to be directed to the production of different categories of product – and subsequently allowing the company to determine whether the yield, quality or safety of a particular category was related to a particular raw material – or a particular ingredient. As traceability systems are basically record-keeping systems, these are in some form required in order for an HACCP system to be implemented. However,

the record-keeping step of the HACCP system aims at documenting that the system is under control, that corrective actions are taken when predefined critical limits are exceeded, and that recall of unsafe products is undertaken when required (Caporale *et al.*, 2001). A fully implemented traceability system is broader and covers also a range of aspects not related to safety.

Finally, implementation of traceability systems, although costly to implement, can also be an economic benefit to the producer. The whole chain from vessel to retailer can be managed in a more effective way, when the traceable information is actively used to enhance mutual trust and cooperation between steps in the chain. Significantly less time (and money) can be spent on quality checks and storage. In addition, when recalls are necessary, traceability gives an assurance that the company limits the loss, and protects its brand on the market (Frederiksen, 2002).

6.7.1 Internal versus external (chain) traceability

The global acceptance of HACCP systems for safety management has increased the need for product chain information throughout the chain (McKean, 2001). Many food (fish) processing companies already have effective internal traceability systems as part of their HACCP based quality assurance systems. In many cases, however, traceability is lost before and after the company deals with the raw materials and the final products. Much effort is spent on quality and safety grading of incoming raw material. This effort can be minimized if the external traceability, the so-called chain traceability, and the attached information on quality are established. The traceable information must be reliable, and this is substantiated by open access from other chain members to audit the quality assurance systems in the chain. Chain traceability is key to cooperation and mutual trust between independent companies in a chain. Developed industries such as the automotive industry focus on auditing their subsuppliers quality assurance systems and make less inspection of incoming products. This is also the case in some food industries.

6.7.2 Traceability systems

Traceability in its simplest form is the existence of a paper trail. This implies that every relevant piece of information is written on paper that follows the raw material through the processing line to retail. This method can be used for products of high value that are only produced in small quantities, but for basic commodity fish products the costs are too high for manual tracking (Frederiksen and Bremner, 2001). Despite the costs, analysis of three different fish chains in Denmark, Iceland and Norway (fresh whole fish, frozen fish and fresh farmed salmon) have shown that the paper-based systems (faxes, notes and postal letters) are widely used (Palsson *et al.*, 2000).

With the explosive development in electronic data analysis, traceability systems based on information technology are being continuously developed (Frederiksen *et al.*, 2002). Several e-business companies produce software allowing integration of financial and production data in one programme package, and most of these have implemented traceability capability components. However, such systems are typically too costly for the small business units in the fish industry.

The EDIFACT (Electronic Data Interchange for Administration, Commerce and Transport) standard is currently the most used standard for transferring data between steps in the chain. Transfer costs are high, and the standard is mostly used by supermarkets at the retail end of the chain. The Internet is the transfer medium of the future, and XML (extensible mark-up language) is a very convenient Internet standard allowing transfer of information in a readable, easy and cheap way (W3C, 2013).

In several projects in the European Union (Member Organization), industry traceability standards have been developed together with methods to analyse, define and transfer information in the seafood sector and recommendations for good

traceability practice (TraceFood, 2013). Tracefish was the first open voluntary industry CEN standard for how traceability may be implemented for farmed and captured fish (CEN, 2003). TraceFood¹⁴ is a website where results of traceability are collected and kept for the future. An important part of the TraceFood framework is the electronic language used for coding and exchanging information about food products in general, called TraceCoreXML. This language has been developed for food businesses that want to send and receive traceability information in a standardized electronic format (Storøy, Thakur and Olsen, 2013).

6.7.3 Product labelling

The minimum requirement for traceability is that each traceable unit has been uniquely labelled to allow identification. The most common labelling method is to label products with standard barcodes from GS1 (Global System one) of which the EAN-13 and UPC-12 codes (European Article Number and Uniform Product Code) are the most used. However, these codes, which can be read by retail units, do not allow inclusion of a unique identifier, which is crucial for traceability. The barcode GS1-128 includes the identifier but cannot be read by the retail bar code scanner without modifications (GS1, 2013). GS1 have also developed a standard based on use of barcodes in the whole chain called “The Traceability of Fish Guideline” (GS1, 2002), which specifies the minimum requirements for ensuring the traceability of fish and fish products with barcodes based on the Tracefish standards.

The newest development is the use of radio frequency identification (RFID) tags, but the price is currently too high to justify their use in the consumer end of the chain. However, they are used today for reusable fish tubs, crates and pallets in supermarket distribution centres and as an internal traceability keeper in the meat industry. The advantage of these tags is that they are fast and easy to read. It must be anticipated that the price of the RFID tags will decrease to a level allowing them to be introduced more widely in the food chain. Standards have also been developed for RFID tags under the term EPCglobal (Electronic Product Code). The EPCglobal standards use the GS1 identification system as a basis together with RFID tags instead of barcodes (EPC, 2013).

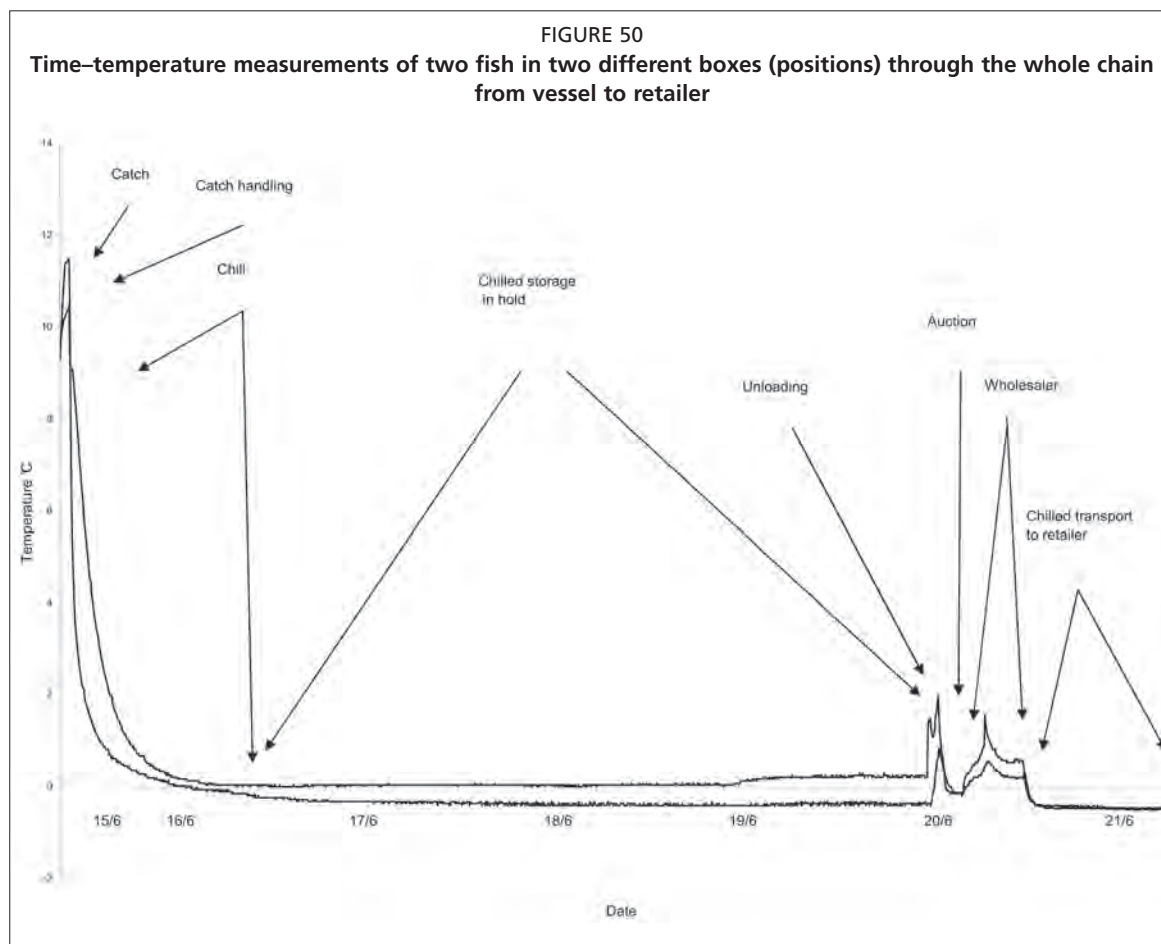
6.7.4 Fresh-fish quality traceability

Traceability is important in the fresh-fish chain where it allows tracing of fish from tropical reef waters (potentially containing marine toxins) or tracing of fish from waters polluted with, for example, heavy metals. However, the most important issue in fresh-fish trading is the assurance of freshness. Freshness – for all species – is almost exclusively a function of time and temperature. In principle, each fish should be continuously monitored with a time–temperature recording device; however, this is not often technically or economically feasible. Therefore, these two aspects are dealt with separately. In a well-functioning distribution chain, where each step can be relied upon in terms of temperature control, quality traceability can be implemented by a time recording. Spot checks on quality must be carried out using standardized fresh-fish quality inspection methods such as the QIM (Bremner, 1985; Jónsdóttir *et al.*, 1991).

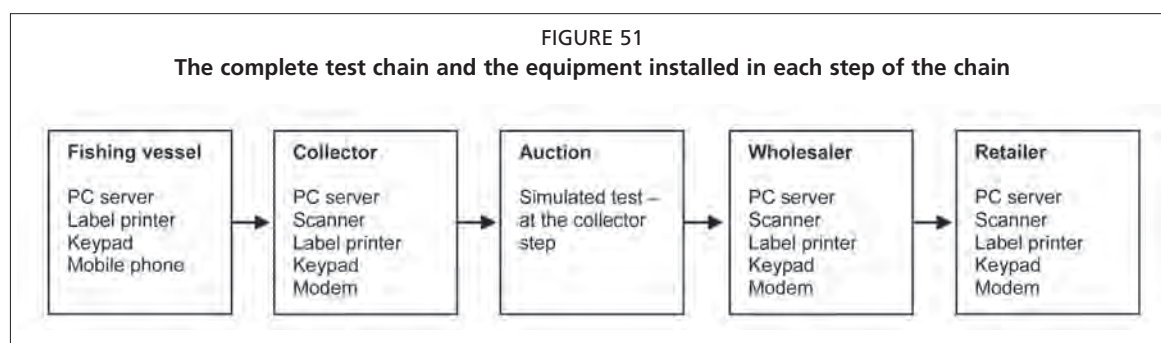
A traceability system has been developed for fresh-fish supply chains in the Danish domestic market, and initial studies have shown that temperature could be controlled appropriately in this particular chain (Figure 50).

Internet technology (XML) has been used to transfer data from the five steps in the chain from fisher to retailer (Figure 51).

¹⁴ www.tracefood.org



Source: Modified from Frederiksen *et al.* (2002).

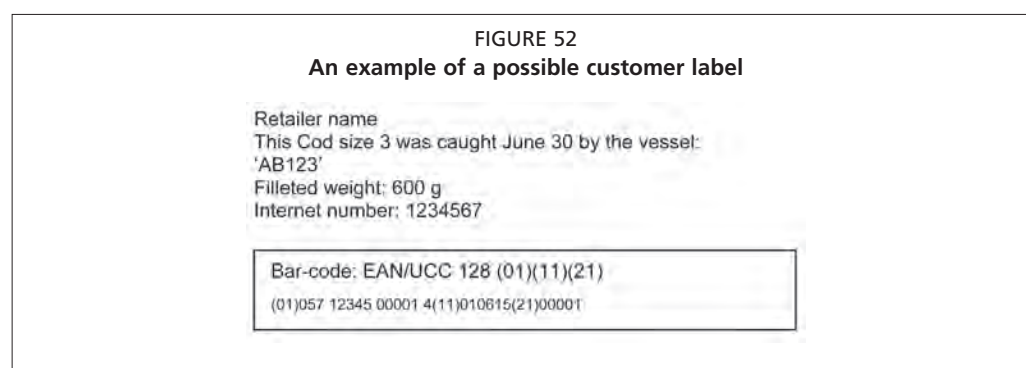


Source: Modified from Frederiksen *et al.* (2002).

Fish are sorted on board according to species, and iced in boxes. Each box is labelled with information on fish species, catch date, vessel name/number, and a unique box number, readable as ordinary numbers and in the form of a barcode. The information is registered in a computer on board the vessel, and the data are transmitted via a mobile phone to a computer at the next step in the chain, the collector. The collector receives all the information from the vessel before it enters the harbour. At the collector, each species is sorted according to size, keeping fish from each catch date separate (the traceable unit is fish from the same vessel with the same catch date). The fish is ice-packed in boxes, with new labels attached, and the information about the collector's

name, fish size/weight and a new box number is registered in the computer, adding this new information to the database.

The boxes are distributed through a wholesaler and further on to a retailer, and the same procedures are used in all steps to retrieve and add new information to existing product data. The information about new fish weights and new box numbers are added to the wholesaler information under the wholesaler name during the repacking process. At the level of the retailer, information on the retailer's name, new fish weight, process type and customer number are added during the sales operation. All the information is available at the retailer step. An example of a possible customer label is shown in Figure 52.



Source: Modified from Frederiksen *et al.* (2002).

6.7.5 Legislation of the European Union (Member Organization) on traceability of fish and fish products

There is great international awareness about the need for traceability. The European White Paper on Food Safety (EC, 2000) and the Bangkok Declaration and Strategy on Aquaculture Development (NACA/FAO, 2000), both include statements encouraging the development of traceability to be applied throughout the supply chain.

The general European Union (Member Organization) principles and requirements of food law, including traceability definition and requirements are contained in the European Union Commission regulations 178/2002 (EC, 2002a). The present legislation for traceability of fish and fish products is described in European Union Council Regulation 104/2000 (EC, 2000b), European Commission Regulation 2065/2001 (EC, 2001b) and European Council Regulation 1224/2009 (EC, 2009a). This regulation states that, at the point of consumer purchase, the following aspects should be documented:

- Species (trade name and/or Latin name).
- Production method ("caught at sea" or "in inland waters" or "farmed").
- Catch area. For fish caught at sea, the FAO catch area must be stated. For fish from inland waters, the country of origin must be given; and for farmed fish, the country of the final processing of the product must be given.
- Whether the fishery products have been previously frozen or not.

The catch area requirement is very broad and currently only requires a distinction between fish from the whole of the North Sea and the Baltic Sea for catches in North Europe. This has far-reaching consequences. If, for example, pollution is detected in a small sea area in the North Sea, then all fish caught from the North Sea must be recalled.

The legislation on traceability in the United States of America is stricter than that in the European Union (Member Organization), with more focus on protecting the food supply from terrorism. The legislation is published in the United States Public

Health Security and Bioterrorism Preparedness and Response Act of 2002 (known as the Bioterrorism Act of 2002) (FDA, 2002). The traceability requirements are specified in the final rule Title 21 CFR Part 1, Subpart J: Establishment, Maintenance, and Availability of Records.¹⁵

More recently, at the Thirtieth Session of the FAO Committee on Fisheries (COFI) in 2012, it was recognized that an integrated approach to the implementation of traceability – for food safety purposes and for control of illegal, unreported and unregulated (IUU) fishing – is important. It was noted that, in developing best practice guidelines for traceability, FAO should be guided by the following principles, in that any guideline:

- should not create unnecessary barriers to trade;
- embraces the concept of equivalence;
- is risk-based;
- is reliable, simple, clear and transparent.

6.8 MONITORING AND SURVEILLANCE PROGRAMMES (LAHSEN ABABOUC AND IDDYA KARUNASAGAR)

In Chapters 3 and 4, several hazards (biotoxins, faecal and chemical contaminants) and quality defects have been associated with the practices and environmental conditions during fishing and aquaculture production. Chapter 3 provides comprehensive overviews for the assessment of risks associated with these hazards. The management of these risks to prevent or control these hazards requires the development and implementation of robust monitoring programmes of the fishing grounds and aquaculture operations. These monitoring programmes are generally enacted through regulations that define responsibilities and resources to food control authorities that will manage the monitoring programmes, although research and industry are also involved.

This section summarizes the requirements of monitoring programmes for live and raw bivalve molluscs, based on the deliberations that have taken place within the framework of the CCFFP (CAC, 2003; Lawrence *et al.*, 2011) and of chemicals and veterinary drugs within the framework of regulations and practices in major markets.

6.8.1 Monitoring of bivalve molluscs

Monitoring is an important tool in the management of food safety for bivalve molluscs, such as oysters, mussels, scallops or cockles. Bivalve molluscs represent about 10 percent of total world fish and seafood production, but 26 percent in terms of volume and 14 percent of total aquaculture production (FAO, 2012).

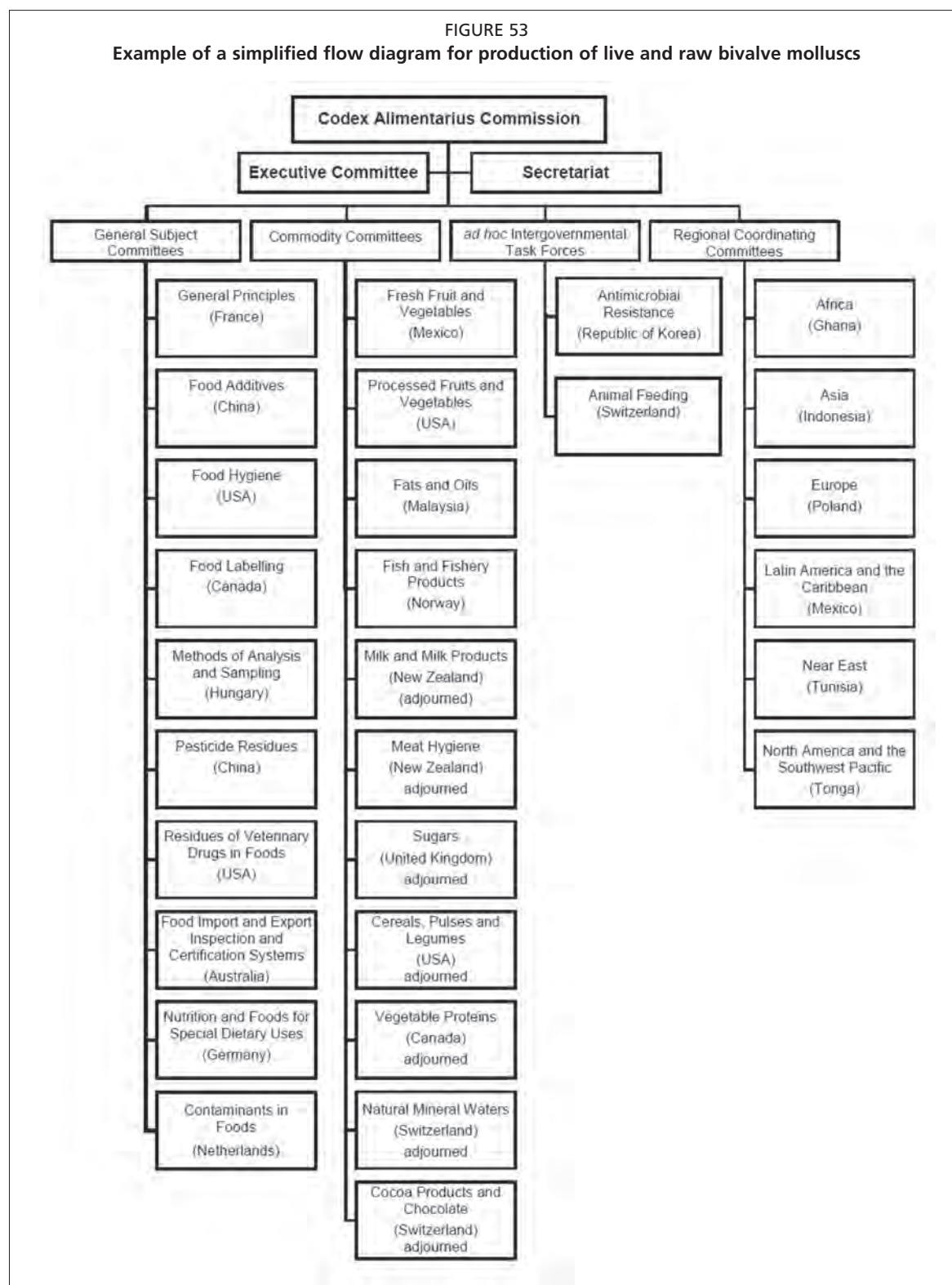
Bivalve mollusc species such as oysters, mussels and clams can survive for extended periods out of water and are traded widely for human consumption as live animals. Other species such as cockles are traded live if carefully handled, but are normally processed. Figure 53 describes a flow diagram for live and raw bivalve molluscs and indicates the primacy of monitoring in the value chain.

The main hazard known for the production of bivalve molluscs is the microbiological contamination of waters in which they grow, especially when the bivalve molluscs are intended to be eaten live or raw. Because molluscs are filter feeders, they concentrate contaminants to a much higher concentration than the surrounding seawater. The contamination with bacteria and viruses of the waters in the growing area is therefore critical for the end-product specification and determines the process requirements for further processing. Gastroenteritis and other serious diseases such as hepatitis can occur as a result of agricultural runoff and/or sewage contamination such as enteric

¹⁵ www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1&showFR=1&subpartNode=21:1.0.1.1.1.8

bacterial and/or viral pathogens (norovirus, and viruses causing hepatitis) or from natural occurring bacterial pathogens (*Vibrio* spp.).

FIGURE 53
Example of a simplified flow diagram for production of live and raw bivalve molluscs



Source: CAC (2003).

The second important hazard associated with the production and consumption of bivalve molluscs is biotoxin presence. Biotoxins are produced by some algae and can cause various forms of serious poisoning such as DSP, PSP, NSP, ASP and AZP.

Chemical substances, such as heavy metals, pesticides, organochlorides, petrochemical substances may also form a hazard in certain areas.

The identification, classification and monitoring of these areas is a responsibility for competent authorities in cooperation with fishers and primary producers. *E. coli*, faecal coliforms or total coliforms may be used as an indicator for the possibility of faecal contamination. Bivalve molluscs from waters subject to microbiological contamination, as determined by the authority having jurisdiction, can be made safe by relaying shellfish in a suitable area or through a depuration process to reduce the level of bacteria, if the process is continued long enough, or by processing to reduce or limit target organisms. Depuration is a short-term process commonly used to reduce low levels of bacterial contamination, but long-term relaying is required if there is a greater risk of contamination.

If biotoxins are found in the flesh of bivalve molluscs in hazardous amounts, the growing area must be closed for harvesting bivalve molluscs until toxicological investigation has made clear that the bivalve mollusc meat is free from hazardous amounts of biotoxins. Harmful chemical substances should not be present in the edible part in such amounts that the calculated dietary intake exceeds the permissible daily intake.

6.8.1.1 *Classification of growing areas*

Surveys of the growing area, shoreline and land catchment should be conducted to determine sources of both domestic and industrial pollution that may affect the quality of the growing water and bivalve molluscs. Sources may include municipal sewage outputs, industrial outputs, mine wastes, geophysical contaminants, domestic-animal holding pens, nuclear power plants, refineries or other sources. The need to reschedule hygiene surveys will be determined by population shifts and changes in agricultural and industrial activities in the coastal area. Regular surveys should be conducted at an acceptable frequency, and known pollution sources should be re-evaluated on a regular basis to determine any changes to their impact on the growing area.

When pollution sources have been identified and evaluated, sampling stations for water and/or bivalve molluscs and/or sediments should be established and studies conducted to determine the effects of the pollutants on the quality of the water and the bivalve molluscs. The data should be evaluated by the official agency having jurisdiction, and growing areas should be classified according to official standards and criteria.

When interpreting growing-area data, the official agency having jurisdiction should take into account variations that may affect the level of pollution during the most unfavourable hydrographic and climatic conditions as influenced by rainfall, tides, winds, methods of sewage treatment, population variations and other local factors, as bivalve molluscs respond rapidly to an increase in the number of bacteria or viruses in their environment by accumulating these agents. The agency should also consider that bivalve molluscs have the ability to accumulate toxic chemicals in their tissue in concentrations greater than the levels found in the surrounding water. FAO, WHO or other international or national food standards may be used as a guide to acceptable levels.

The official agency having jurisdiction should immediately announce decisions concerning the classification of growing areas to the affected producers and depuration and distribution centres.

When sampling shellfish meats for classification purposes, if the limits of any biological or chemical hazard set in the end-product specification are exceeded,

appropriate measures must be taken under the responsibility of the official agency having jurisdiction.

Classified growing areas should be clearly defined by the official agency having jurisdiction as:

- suitable for harvesting for direct human consumption, relaying in acceptable water or depuration in an approved depuration centre or approved processing to reduce or limit target organisms; or
- non-suitable for growing or harvesting bivalve molluscs.

6.8.1.2 *Monitoring of growing areas*

Factors affecting the occurrence and accumulation of toxic algae cannot be controlled and the prediction of toxic algae has severe limitations. Growing areas should be routinely monitored for changes in water quality and/or bivalve mollusc quality and substandard areas patrolled to prevent harvesting for purposes other than that established by the official agency.

Biotoxins in bivalve molluscs can be caused by plankton containing toxins. For early warning purposes, where appropriate, it is recommended to have a programme to monitor growing areas for the species of plankton that can produce toxins and to recognize other environmental signals that a toxic event may be developing.

Harmful chemical substances within bivalve molluscs should not be present in amounts such that the calculated dietary intake exceeds the permissible daily intake. A monitoring system should be present for harmful chemical substances.

When routine monitoring programmes or re-surveys show that the growing area no longer meets the classification criteria, the area should be reclassified or closed for harvesting immediately by the official agency having jurisdiction.

In determining the public health suitability of bivalve mollusc classified growing areas, the official agency having jurisdiction should consider the following actions:

- Classification/reclassification of growing areas by sanitary survey, monitoring of *E. coli* / faecal coliforms or total coliforms at an appropriate frequency based on the risk of contamination, and other sanitary control measures as applicable.
- Classification/reclassification of growing areas by monitoring of pathogens at an appropriate frequency based on the probability of contamination in bivalve mollusc meat.
- Closure/reopening of growing areas by the monitoring of biotoxins in bivalve molluscs alone or in combination with the monitoring of phytoplankton in seawater at an appropriate frequency based on the probability of contamination.
- Control of chemical contaminants.

Under the responsibility of the official agency having jurisdiction, the growing areas providing bivalve molluscs for direct human consumption must meet the following requirements at the time of harvest:

- The area is not subject to contamination that may present an actual or potential hazard to human health.
- The bivalve molluscs harvested meet the end-product specification. This can be determined by examination of mollusc flesh or through adequate monitoring of the water, as appropriate.

Growing areas providing bivalve molluscs for indirect human consumption should be defined in relation to the further procedure of the lot.

6.8.1.2.1 *E. coli, faecal coliforms and total coliforms*

All growing water and/or molluscan flesh should be monitored for the presence of *E. coli* / faecal coliforms or total coliforms at an appropriate frequency based on the probability and degree of faecal contamination. Tests for suitable indicator bacteria

such as faecal coliforms or *E. coli* or total coliforms should be used to determine the degree of faecal contamination. The effectiveness of indicator bacteria used should be kept under constant review for their reliability as measures for the degree of faecal contamination. If faecal contamination exceeds certain threshold-levels, relaying or depuration for a time approved by the official agency having jurisdiction may be allowed.

E. coli / faecal coliforms or total coliforms may be used as an indicator for the presence of faecal contamination. Because these indicators do not correlate well with the presence of viruses, other controls such as shoreline surveys should always be employed.

Other methods such as bacteriophage and viral detection could also be used as indicators when validated analytical methods become available in the future.

6.8.1.2.2 Pathogen monitoring

Shellfish sanitation programmes rely upon the use of indicator organisms for the presence of contamination rather than upon attempts to monitor for specific pathogens. However, where there has been a shellfish-borne outbreak caused by an identified pathogen such as *Salmonella* and others (*Vibrio* and viruses), monitoring the bivalve molluscs may be appropriate as part of the process of closure/reopening the affected harvest area. The species, and typically the actual strain, should be known to ensure that monitoring is addressing the source of the pathogen. Predetermined acceptance/rejection levels for the pathogen should have been established in order to use such monitoring results for decision-making. Other conditions including the sanitary survey requirements should also have been satisfied as a condition of reopening this area.

6.8.1.2.3 Marine biotoxin control

Phytoplankton monitoring is a valuable complementary tool that can be used, in combination with the required monitoring of marine biotoxins in shellfish tissue, to optimize programme management and resources.

Growing areas should also be monitored for environmental signals that a toxin event may be occurring, e.g. dead or dying birds, mammals, or fish. The risk of blooms of toxic algae may show seasonal variability, and areas may also be affected by toxic algae previously unknown in the surrounding sea or coastal waters. These risks should be recognized when drawing up monitoring schedules.

It is important to note that, in using indicator shellfish species, the absence of toxicity in indicated species is assumed to imply the absence of toxicity in other species in the growing area. This implication must be verified for each shellfish species and for each group of toxins before defining a particular shellfish species as an indicator for that growing area.

The official agency having jurisdiction should immediately close and effectively patrol affected areas when acceptable levels are exceeded in edible portions of bivalve mollusc meat. These areas should not be opened before toxicological investigation has made clear that the bivalve mollusc meat is free from hazardous amounts of biotoxins.

The official agency having jurisdiction should immediately announce these decisions to the affected producers and depuration and distribution centres.

In establishing sampling programmes over space and time, consideration should be given to ensuring the adequate location and number of sampling sites. Testing for a particular biotoxin may not be appropriate when it has been demonstrated that this biotoxin has not been associated with bivalve molluscs in the growing and harvesting areas. Sampling frequency must be sufficient to address spatial-temporal changes in microalgae, toxins in shellfish and to cover the risks of rapid rises in shellfish toxicity.

6.8.1.2.4 Spatial representational sampling

The selection of sampling stations for both benthic and suspended culture should be based on sites that have historically presented toxicity in the early stages of a toxic event. It is recognized that sampling, generally, cannot be carried out in a statistically valid way without excessive cost. In order to protect public health, the selection of sampling stations should give appropriate coverage of the extent of a toxic event or the likely “worst case scenario” in a growing area. This should be based on expert judgement using the following factors:

- hydrography, known upwellings, fronts, current patterns and tidal effects;
- access to sampling stations in all weather conditions during harvesting;
- desirability of toxin and microalgal sampling at the same sampling station;
- in addition to primary (routine) stations, the need for secondary (complementary) and offshore stations;
- existence of *in situ* growth (for example, toxic microalgae from cyst beds);
- the advection of offshore toxic microalgal blooms into growing areas.

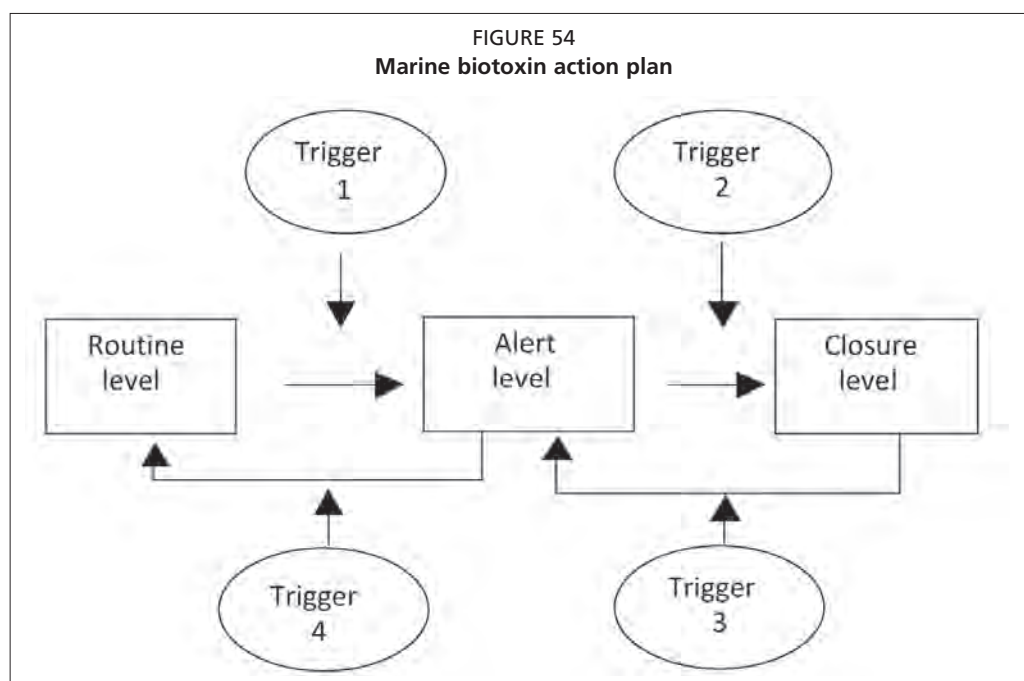
Routine sampling for microalgae will generally mean taking an integrated sample from the water column. When a toxic event is in progress or developing, targeted, depth-specific sampling should be considered.

Sampling for shellfish grown in suspension should, at the very least, involve an integrated sample composed of shellfish taken from the top, middle and bottom of the lines.

6.8.1.2.5 Temporal representational sampling

Minimum weekly sampling frequencies are adopted by most monitoring programmes in areas where toxicity is prevalent and where harvesting is taking place or about to take place. Decisions on the frequency of sampling should be based on risk evaluation. Inputs into the decision may include factors such as seasonality (toxicity and/or harvesting), accessibility, historical baseline information, including toxin and microalgal data, and the effects of environmental factors such as wind, tide and currents.

The sampling frequency and the factors that may lead to it being changed should be described in a “marine biotoxin action plan” for the growing area (Figure 54).



6.8.1.2.6 *Shellfish sample size*

There is no internationally agreed sample size for different shellfish species. There may be high variability of toxicity among individual shellfish. The number of shellfish sampled should be sufficient to address this variability. For this reason, the number of shellfish in the sample, rather than the mass of the shellfish flesh, should be the determining factor for the sample size. In addition, the size of the sample should be sufficient to allow the test or tests for which the sample is being taken to be carried out, and the shellfish sampled should be of the size marketed.

6.8.1.2.7 *Marine biotoxin test methods*

A method suitable for the determination of the saxitoxin group of marine biotoxins is provided in the Codex Standard for Live and Raw Bivalve Molluscs (CAC, 2008a). As internationally validated methods are not available for other biotoxins, currently, the CCFFP is working on performance criteria of methods to be used for these toxins. Any methods may be deemed suitable for screening purposes provided they are approved by a country's competent authority.

6.8.1.2.8 *Chemical contaminants*

Growing areas should be monitored for chemical contaminants on a sufficiently frequent basis to provide confidence that any identified sources of chemical contamination are not contaminating the shellfish.

Shellfish-growing areas where there are no known point sources of likely chemical contamination should only require occasional checks every few years. However, where there are known point sources of specific contamination, shellfish may need to be checked more frequently on a routine basis. There should also be the capacity to sample shellfish reactively if a defined event occurs – for example, a spillage of anti-fouling paint.

Chemical contaminants are a concern not only for bivalve molluscs but also for other aquatic animals (fish, crustaceans and cephalopods) harvested in freshwater, estuaries and coastal waters where shore-side industries are located or intensive agriculture using pesticides or other agrochemicals is practised. In these cases, a monitoring programme is also required.

6.8.2 Monitoring fish and fishery products for chemical contaminants and residues of veterinary drugs

Modern food safety control programmes are based on the principles of risk analysis. As indicated in Section 2.4, risk analysis has three major components: risk assessment, risk management and risk communication. It has also been pointed out that risk management starts with risk evaluation, which includes the identification of food safety issue and the development of a risk profile. In the case of microbial hazards, a food safety issue may be brought to the attention of risk managers because of an outbreak of food-borne infection. With microbial hazards, most adverse affects are acute and the result of a single exposure event e.g. a meal of contaminated food. With chemical hazards, such as pesticides and heavy metals, adverse health effects are caused by the cumulative effect of multiple exposures. In the case of microbial hazards, the level of the micro-organism may go up or down in the food chain and even contamination may take place at various stages of food chain, while in the case of chemical hazards, such as pesticides, residues of veterinary drugs or heavy metals, they are present at the primary production stage and their levels are not altered along the food chain. Therefore, in order to perform a risk evaluation, it is important to have information on the presence of the chemical hazard at the primary production stage.

Control of microbial hazards involves implementation of measures in food chain, and the responsibility lies with those involved in handling and processing of food. On

other hand, control of chemical hazards involves identification of fishing grounds or fish farms where levels of hazards are above acceptable limits and, generally, this involves monitoring, testing and implementing control measures to minimize the public health risk, and this is generally the responsibility of national regulatory agencies.

6.8.2.1 *Environmental monitoring in the United States of America*

Environmental monitoring can identify species susceptible to contamination, the magnitude of contamination and the spatial distribution of contamination. Information obtained by monitoring could be used by the competent authorities to develop fish advisories for consumers, as is done in the United States of America.

The United States Environmental Protection Agency (EPA) has developed guidance for assessing chemical contaminant data for use in fish advisories. Volume 1 of the National Guidance¹⁶ deals with fish sampling and analysis. This could be a useful guide for the development of national fish-contaminant monitoring plans in other countries. It recommends a tiered approach, where tier 1 involves screening of a large number of sites to identify areas where the concentration of contaminants in edible fish tissue indicates the potential for a significant health risk to fish consumers. If problem areas are identified in the screening studies, a two-phase intensive study (tier 2) is performed, where phase 1 involves determining the magnitude of contamination in commonly consumed fish and shellfish species, and phase 2 involves determining the geographical extent of the contamination and the size-specific level of contamination. This tiered approach would make monitoring cost-effective, because the screening studies would help by limiting the sites to be subjected to intensive study and limiting the target analyses at each intensive sampling site. Nevertheless, the public health objectives would be met. For both screening as well as intensive study, target fish species need to be chosen to include commonly consumed fish species that are known to bioaccumulate contaminants and are distributed over a wide geographic area. Generally, one bottom feeder and one predator are chosen as target species. While choosing sampling sites, consideration should be given to fishing areas that have a high probability of contamination and presumed clean sites. Samples consist of edible portions of fish and, where possible, composite samples are taken for analysis using standard methods.

The Codex Standard Codex Stan 228-2001 General Methods of Analysis for Contaminants provides guidance on methods to be used for the analysis of heavy metals. Figure 55 provides an overview of the steps involved.

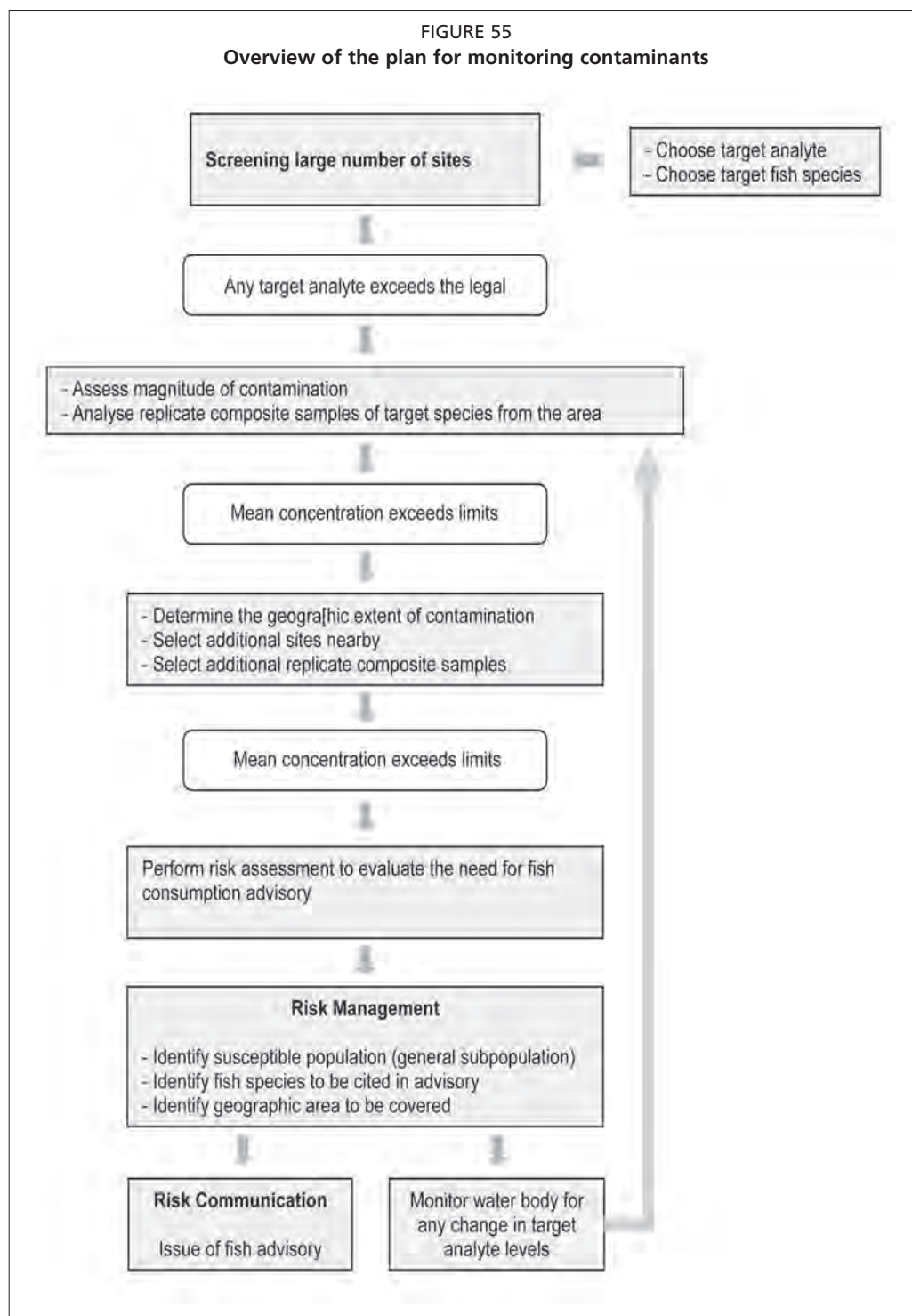
6.8.2.2 *Regulations of the European Union (Member Organization) on monitoring for environmental contaminants*

In the regulations of the European Union (Member Organization), monitoring primary production areas for environmental contaminants has been included as a part of food safety management. There are several relevant regulations and directives:

- Regulation 1881/2006 (EC, 2006a) lays down maximum levels of contaminants (heavy metals, dioxins, PCBs, polycyclic aromatic hydrocarbons).
- Directive 96/23 (EC, 1996a) deals with residue monitoring in aquaculture products.
- Regulation 1883/2006 (EC, 2006b) deals with sampling methods and methods of analysis for dioxins and PCBs.
- Regulation 333/2007 (EC, 2007b) deals with sampling methods and methods of analysis for heavy metals.
- For pesticides, MRLs are laid down in Directive 86/363/EC (EC, 1986) and its amendments.

¹⁶ <http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/>

A guide for the establishment of environmental and residue monitoring plans for compliance with regulations of the European Union (Member Organization) has been developed under the project of the European Union (Member Organization) Strengthening Fishery Products – Health Conditions in ACP/OCT Countries.¹⁷



Source: Modified from the strategy of the United States Environmental Protection Agency.

¹⁷ http://sfp.acp.int/sites/all/files/tmp/07_07_ACP_EMP_RMP_Guide_EN_2.pdf

Third countries wanting to export fishery products to the European Union (Member Organization) should have a national residue control plan (NRCP), and the competent authority should designate the person responsible for the implementation of the NRCP. The development of a sampling and analysis plan should be based on the knowledge of the fishery and the likely sources of contamination (e.g. location of the outfall from industries such as tanning and metal finishing). Baseline information could be obtained from bibliographic sources. A team consisting of government departments, research institutions and the fishery industry may be involved in the development of the sampling plan.

In capture fisheries, the contaminants of interest are heavy metals (arsenic, lead, mercury and cadmium); polycyclic aromatic hydrocarbons (benzo- α -pyrene being the marker compound); persistent organic compounds (PCBs, organochlorine pesticides and dioxin-like compounds). In each area, the target fish need to be identified. These could include predators, bottom feeders, shellfish, other species susceptible for contamination, and species intended for export, etc. Sampling options and decisions are made based on the risk of contamination.

The monitoring plan should specify the type of sample (whole animal, edible portions, etc.), the number of samples, the location of the sampling, the target contaminants to be analysed, the analytical laboratory where the analysis will be performed, how the data will be presented (e.g. concentration, wet weight, dry weight), the analytical time frame and the mode of reporting.

Sampling is to be done at the point where the commodity enters the food chain, e.g. when landed. Incremental sampling is prescribed in Regulation EC/333/2007 (EC, 2007b) Part B, for example, for a lot of < 50 kg, a minimum of 3 samples are to be taken and this increases to 5 samples for 50–500 kg, and 10 samples for > 500 kg. Samples are normally chilled during transport, and freezing can result in loss of water content upon thawing that would invalidate the results. The frequency of sampling could be decided by the competent authority based on risk, for example, on an annual basis for susceptible species in areas with no history of contamination.

The laboratory should use validated methods of analysis, observe the performance criteria for the analysis and provide the analytical results in the same units and with the same number of significant figures as the maximum levels laid down in Regulation EC 1881/2006 (EC, 2006a). Alternative methods that are not fully validated but are suitable for official control must meet the “fitness for purpose” approach. Where a limited number of validated methods of analysis exist, it may be possible to use a “fitness for purpose” approach to assess the suitability of the method of analysis. Methods suitable for official control must produce results with standard measurement uncertainties less than the maximum standard measurement uncertainty that is calculated using the formula set out in EC 333/2007 (EC, 2007b).

When contaminants above permissible limits are found, the risk management measures to be taken are: (i) trace the source of contamination; (ii) define the affected area and map the boundaries; (iii) suspend fishing in affected areas; and (iv) review the status with further sampling and analysis.

In the case of aquaculture, residue monitoring is used to verify that when pharmacologically active substances are used to treat disease, the use is done in accordance with national regulations and international guidelines, appropriate withdrawal periods are followed, and the residue levels are within limits, where MRLs exist. In the European Union (Member Organization), the use of pharmacologically active substances in food-producing animals is regulated by the Council Regulation EEC 2377/90 (EC, 1990) and the substances are placed in four Annexes in this regulation:

- Annex I: Substances for which full residue evaluation has been performed and MRLs elaborated.

- Annex II: Substances for which full residue evaluation has been performed and no MRLs are considered necessary.
- Annex III: Substances for which some residue data are available and temporary MRLs have been established pending full evaluation.
- Annex IV: Substances which are prohibited for use in food producing animals, e.g. chloramphenicol and nitrofurans.

Regarding monitoring, Council Directive 96/23/EC (EC, 1996a) lists groups of substances to be tested in food-producing animals:

- Group A includes thyrostatic, gestagenic and beta-agonistic substances that are not applicable to aquaculture. This group also includes oestrogenic and androgenic substances that may be used in aquaculture for sex inversion at early stages (up to three months), but their use for growth promotion is prohibited. Banned antimicrobials such as chloramphenicol and nitrofurans are also in this group.
- Group B includes approved veterinary medicines for which MRLs exist.

Sampling aquaculture sites for monitoring could be either (i) targeted, where sampling points are selected based on results of previous monitoring results or evidence from inspection, or (ii) random. It is important that sampling points be selected to represent different aquaculture species and different regions in the country. According to the regulations of the European Union (Member Organization), at least one sample should be tested for 100 tonnes of production, but more samples may be required to cover different species and regions. Sampling should be systematic, representative, documented and determined in advance. Sampling may coincide with other activities, e.g. inspection of a facility/audit, but the timing needs to be distributed evenly during the culture period.

Screening methods such as ELISA may be used, but positive results need to be confirmed using methods such as LC-MS/MS. For banned antibiotics such as chloramphenicol and metabolites of nitrofurans, the method to be used should be able to achieve the set MRPL. In the European Union (Member Organization), the MRPL for chloramphenicol residue is 0.3 ppb, and 1.0 ppb for metabolites of nitrofurans (EC, 2003).

Results of residue monitoring should be presented along with the information on the maximum levels permitted in the national regulation. In many countries producing aquaculture products for export, it is common to adopt international MRLs and MRPLs in the national regulation. In Codex, MRLs exist only for tetracycline in fish and shrimp (CAC, 2012). There are instances of different MRLs for the same antibiotic in different fish-importing countries. For example, for florfenicol, the MRL in finfish as per Regulation No. 37/2010 of the European Union (Member Organization) is 1 ppm (EC, 2010), but in Japan, the MRL is 0.2 ppm in salmon and trout, 0.03 ppm in marine fish (seabass, seabream and tuna) and 0.1 ppm in crustaceans.¹⁸

When non-complaint samples are found, the competent authority has the responsibility to conduct investigations at the farm to identify the cause/source of contamination and the extent of stock that is contaminated. This could involve verification of records and re-sampling. In the case of MRLs exceeding those for permitted antibiotics, the farm may be quarantined and the grow-out period extended until retesting demonstrates that permitted levels have been achieved. In the case of detection of residues of banned antibiotics, the products are to be disposed of in such a way that they do not re-enter the food chain.

¹⁸ From the website of the The Japan Food Chemical Research Foundation:
www.m5.ws001.squarestart.ne.jp/foundation/agrdtl.php?a_inq=68300

6.9 PRIVATE STANDARDS AND CERTIFICATION SYSTEMS (LAHSEN ABABOUC, SALLY WASHINGTON AND JOHN RYDER)

6.9.1 The emergence of private standards and certification for fish and seafood safety and quality

As mentioned in Chapter 1, increasing amounts of fish and seafood are now caught in one part of the world, transported to another for processing and finally consumed in yet another. Therefore, food safety systems that function across national borders are vital.

A range of national and international regulatory frameworks has been developed accordingly. These are described in Chapter 7. Despite these international frameworks and attempts to harmonize requirements and conformity assessment procedures, fish exporters still face safety and quality control regimes that vary from one jurisdiction to the next. Even within the European Union (Member Organization), where the goal is to harmonize food safety regulations, differences in national regulations still exist for several issues. The United States of America has its own particular requirements, as do other key import markets such as Japan, the Russian Federation and China. This multitude of approaches imposes significant compliance costs¹⁹ on exporters, particularly those in developing countries where there is limited capacity to develop comprehensive safety and control infrastructures, let alone several different systems to meet diverse import market requirements.

Further complicating the variety of public-sector food safety regulations is the multitude of standards applied by the private sector. These relate to a range of objectives, including food safety and quality but also to animal health, environmental protection and even social development, and they are often linked to private firms' corporate social responsibility strategies.

A range of factors has fuelled the trend towards private safety and quality standards. Food safety scares have weakened public confidence in governments' abilities to guarantee food safety, especially the safety of imported food. Government policies related to product liability and due diligence as well as the shift towards more performance-based regulatory frameworks have put the onus on private sector firms to assume responsibility for food safety management. Large food firms, especially retailers, have increasing bargaining power *vis-à-vis* other businesses in the supply chain, and are requiring suppliers to be certified to private food safety management schemes (FSMSs).

Private standards provide buyers with some insurance against food scares and a due diligence defence. Third-party certification offers buyers direct access to written audit reports and/or their results. In contrast, certification by competent authorities (government inspection agencies) and their compliance conformity evaluations are targeted at providing assurance to other public control authorities, not individual private-sector buyers. Publicly available results might only be presented in the aggregate to give assurance that the overall system is functioning well.

The increasing vertical integration and complexity of supply chains in fish and seafood also stimulate the growth of private standards, as business-to-business tools used in the context of direct procurement contracts, which are starting to replace the traditional structure of "importer-wholesaler-retailer". Complex value chains – where raw materials are potentially sourced globally, processed in a second country and retailed in yet another – require sophisticated systems for ensuring traceability and guaranteeing that sanitary and hygiene standards are maintained at every stage of the value chain (from farm/boat to fork). These traceability and chain-of-custody systems are built into the frameworks included in most private standards schemes.

¹⁹ Costs also include detentions and rejections of products deemed not to be in compliance with importing countries' requirements.

Private safety and quality standards related to fish and seafood apply to both wild capture and farmed fish. A number of private standards schemes specific to aquaculture have also emerged in the past decade that cover the entire supply chain. Most aquaculture certification schemes include multiple standards criteria (safety, quality, animal health, environment and social) and are used to market farmed fish as a safe, sustainable and environmentally sound alternative to fish and seafood from dwindling marine capture stocks. As noted above, aquaculture now accounts for almost half of the fish available for food supply. Private standards are a mechanism for responding to concerns about aquaculture by offering guarantees related to quality, safety, environmental impacts, traceability, and transparency of production processes.

6.9.2 Types of private safety/quality standards in fisheries and aquaculture

In addition to national and international food safety and quality regulations or management systems, there are many different private safety and quality standards applying to fisheries and aquaculture. These include: private in-house standards (producers' or processors' manuals of standard quality operating procedures); buyer guidelines; collective private quality standards (codes of conduct or codes of practice) developed by local, regional or national producer/industry groups; NGO-driven schemes; and national and international FSMSs.

The following section gives an overview of the various types of standards, including illustrative examples. It is organized as follows:

- private in-house standards (guidelines) of large retail firms;
- collective private standards (codes of conduct) developed by local, regional or national producer/industry groups;
- public certification schemes;
- NGO-driven schemes (mainly related to aquaculture);
- national and international FSMSs.

6.9.2.1 Private in-house guidelines of large retail firms

Setting product and process specifications and requiring suppliers to meet those specifications is not a new phenomenon. Most large retailers, as well as large processors and catering firms, have developed their own detailed product and process specifications. Most take mandatory national food safety regulations as a baseline (and, in the case of retailers in the European Union (Member Organization), those issued by that organization) and then build on other specifications in line with their in-house standard operating procedure (SOP). These additional requirements are typically related to quality rather than food safety. Industry sources suggest that they are less likely to include more stringent safety-related criteria than required by national regulations, such as "use by" dates or more stringent requirements in terms of acceptable levels of pathogens (e.g. *Salmonella*) or contaminants (such as heavy metals). However, they usually include stringent SOPs or requirements for certification to a FSMS, which include detailed traceability and audit requirements and documentation.

Retailer product specifications are usually treated as confidential as they are considered commercially sensitive in what is a highly competitive market. However, the package of specifications is likely to include detailed:

- product specifications: organoleptic/sensory/taste, metrological (size, block, dimension, etc.), chemical and physical, bacteriological;
- packing and packaging, labelling requirements;
- delivery conditions (where, when, how much);
- demands for information about the supplier company's safety and sanitary management capacities: SOP, safety and quality management process (including details on product controls), traceability and recall procedures.

These specifications are typically communicated to the next level down in the supply chain – to processors, brokers or importers, which subsequently translate those specifications to their suppliers.

The practice of buyers inspecting suppliers' facilities and auditing their FSMSs has occurred for decades in relation to processed (frozen and canned) fish products. Some retailers and food services are now buying directly from producers and, therefore, communicating specifications directly to them. Many have their own audit and inspection requirements. For example, Carrefour, the world's second-largest retailer in terms of revenue, buys shrimp directly from farmers in Thailand. This involves Carrefour sending its own inspectors to verify that products and farming practices meet its own standards.²⁰ In the United States of America, Whole Foods Market²¹ has developed its own standards for a range of farmed fish and seafood. The standards require that all documentation, records, farms and processing plants be subject to annual inspection (both announced and unannounced spot inspections) by independent third-party auditors selected by Whole Foods Market. Suppliers are required to meet the costs of these third-party audits.

However, rather than develop their own certification and verification schemes, most large retailers, commercial brand owners and food service industry firms prefer to align themselves to (and require suppliers to be certified to) private standards schemes developed by other bodies. Therefore, in addition to their firm-specific product and process specifications, firms might also require their suppliers to be certified to:

- For processed fish and seafood: a national or international food safety management scheme, such as the British Retail Consortium (BRC), International Food Standard (IFS), Safe Quality Food (SQF) (all described below). For example, many UK, North American and European retailers rely on certification to the appropriate BRC Global Standard when doing business with suppliers.²²
- For aquaculture: to one or other of the schemes that merges quality and safety with environmental protection, animal health and even social development. For example, Wal-Mart require farmed seafood to be third-party certified as sustainable using Best Aquaculture Practices of the Global Aquaculture Alliance (GAA) or equivalent standards.²³ Darden Restaurants, the largest casual dining restaurant company in the United States of America, also has a goal that all its suppliers of aquaculture products are certified to GAA standards.²⁴

Requiring suppliers to conform to the firm's own quality and safety standards and/or requiring certification to a FSMS offers assurances of quality, safety and traceability; in short, an insurance policy to protect the value of the firm and its brand.

Adherence to these and a range of other private standards (related to environmental protection, animal health and social development) usually forms part of firms' "corporate social responsibility" strategies, which are marketed both to other businesses as well as to consumers to enhance the firms' overall reputation.

Safety and quality requirements are supported by multilayered audit and inspection requirements. Independent private certification schemes are attractive to large-scale buyers. Requiring third-party certification is cost-effective as it can reduce the need for companies to carry out their own inspection and audit of suppliers.

²⁰ Vanich Sowanapreecha, quoted in "Carrefour leading trend to buy shrimp direct from farmers" *IntraFish*, 7 October 2008.

²¹ www.wholefoodsmarket.com/products/aquaculture.php

²² www.brcglobalstandards.com/GlobalStandards/About.aspx

²³ <http://corporate.walmart.com/global-responsibility/environment-sustainability/sustainable-seafood>

²⁴ www.darden.com/sustainability/default.aspx?lang=en&page=plate§ion=seafood-stewardship

However, large retailers and food firms may not be equally demanding of all their suppliers or product lines. The pressure on suppliers to conform to stringent private standards depends on the market and the type of product in question. For example, requirements are more stringent for private-label and high-risk processed fish and seafood products than for basic commodity fish and seafood.

6.9.2.2 *Collective private standards developed by regional or national industry organizations*

Discussions about private standards usually centre on standards imposed by retailers or other food firms on suppliers further down the supply chain. However, industry organizations have, for years, developed standards schemes as self-imposed specifications or codes of practice. The motivation is to:

- establish quality criteria and diffuse them throughout the local industry (standards creation and implementation);
- promote those good practices as indicators of quality to buyers. Quality assurance is verified through inspection and certification.

Wild-capture seafood quality schemes have usually emerged at the local or regional level. They operate as business-to-business tools aimed at reassuring buyers of the quality of products. Two examples are given below.

SIGES (Sistema Integrado de Gestión) – Salmon Chile: The SIGES standard was developed for the Chilean salmon producers association, Salmon Chile. It is managed by INTESAL, the institute for salmon technology in Chile, and functions as a certifiable integrated management system, dealing with:

- food safety and quality management;
- environmental management;
- occupational safety.

It incorporates all relevant legislation, plus technical standards, and is based on international norms and standards including ISO 9001 and ISO 14001²⁵ and OSHAS 18001.²⁶

The Scottish Salmon Producers' Organisation (SSPO): The SSPO is the trade association for the salmon farming industry in Scotland, the United Kingdom of Great Britain and Northern Ireland, whose membership accounts for 95 percent of the tonnage of Scottish salmon production. It has developed a Code of Good Practice for Scottish Finfish Aquaculture²⁷ that includes compliance points covering: food safety and consumer assurance issues (traceability), fish health and biosecurity, environmental management, fish welfare and care, and feed requirements (including the sustainability of sources of fish used as fish feed).

6.9.2.3 *Public certification schemes*

Although the focus of this chapter is on private standards for safety and quality, it should be noted that a number of public certification schemes have also been developed. Label Rouge is a well-established French quality label (albeit not exclusively related to fish and seafood). Other examples – such as Thai Quality Shrimp – are described below in relation to governments' responses to demands for certified fish and seafood. Most relate to aquaculture.

²⁵ ISO 14001 deals with environmental management systems. See: www.iso.org

²⁶ www.bsigroup.com/en-GB/ohsas-18001-occupational-health-and-safety/

²⁷ www.thecodeofgoodpractice.co.uk/

6.9.2.4 NGO-driven standards and certification – aquaculture

Non-governmental organizations have also been active in developing private standards and related certification schemes, specifically for farmed fish and seafood. These schemes have been born out of a desire to improve the image of farmed fish and seafood as a safe and sustainable alternative to wild capture fish, and are aimed at improving practices generally throughout the industry, including reducing negative environmental impacts. Most of the work to improve management practices has been carried out on salmon and shrimp, mainly owing to their high commodity value and importance as the most-traded fish and seafood products.

Global Aquaculture Alliance (GAA): The certification scheme developed by the GAA is a significant aquaculture scheme in terms of volume and global coverage. The GAA first developed a voluntary best practice programme for aquaculture producers. The Responsible Aquaculture Program included various guiding principles, codes of practice and best practice standards. Responding to industry calls for more formal recognition of these practices, it aligned with the Aquaculture Certification Council, a non-governmental body based in the United States of America, to develop a certification of aquaculture production processes. The GAA's Best Aquaculture Practices (BAP) Standards are applied in a certification system that combines site inspections and effluent sampling with sanitary controls and traceability. Certified producers are entitled to use the "BAP certification mark", a label attached to products from certified fish farms. Standards cover a range of considerations, including: food safety, traceability, animal welfare, community and social welfare and environmental sustainability. Both farms and processing facilities can be certified.

GLOBALG.A.P.: EurepGap, was developed in 1997 by the Euro-Retailer Produce Working Group (Eurep), a private-sector body driven by a group of European retailers. In late 2007, it changed its name to GLOBALG.A.P. to reflect its more international focus. EurepGap was initially designed as a standard for GAPs. Its food safety criteria are based on the HACCP system.

Originally applying to fruits and vegetables, Eurep-Gap was later extended to fish farming practices. It was the first to develop an integrated aquaculture assurance standard (in late 2004). In addition to the general code of practice, specific criteria have also been developed for salmonids, tropical shrimp, *Pangasius* and tilapia. Its aquaculture standard²⁸ covers the entire production chain, from broodstock, seedlings and feed suppliers to farming, harvesting, processing and post-harvest handling operations. It serves as a practical manual for any aquaculture producer, ensuring food safety, minimal environmental impact and compliance with animal welfare and worker health and safety requirements.

It is of particular interest in developing countries because it now offers localg.a.p., which is a stepping stone towards certification and includes the aquaculture sector. GLOBALG.A.P. has strong support in the retail sector in Europe and elsewhere. For example, in the United Kingdom of Great Britain and Northern Ireland, Sainsbury's requires that all fresh produce are sourced from suppliers who are certified to GLOBALG.A.P standards.²⁹

Aquaculture Stewardship Council (ASC): The ASC is an independent not for profit organisation. The ASC was founded in 2010 by WWF and the Dutch Sustainable Trade Initiative to manage the global standards for responsible aquaculture. The ASC programme is open to all fish farms, regardless of their size or location. Small fish farm can make use of support programmes from, among others, the Sustainable Trade Initiative or the World Wildlife Fund.

²⁸ www.globalgap.org/uk_en/for-producers/aquaculture/

²⁹ www.j-sainsbury.co.uk/extras/faqs/responsibility/sourcing/fresh-produce-high-quality-farms/

At present, five standards have been developed and finalised for tilapia, pangasius, abalone, bivalve shellfish and salmon. A shrimp standard is planned for completion in 2013. In the future, more standards for other species will be developed.

Other NGO standards and certification schemes: Friend of the Sea (FoS) was set up in 2006 with origins in the Earth Island Institute. It covers both wild capture and farmed fish and seafood with an environmental focus.

Other niche markets, such as organic aquaculture, have also been developed. There are about 20–25 certifying bodies for organic aquaculture products. For example, Naturland,³⁰ based in Germany but operating internationally, certifies organic farmed seafood. However, organic aquaculture accounts for very small volumes of production – only about 1 percent of overall aquaculture production.

6.9.2.5 *Private food safety management schemes*

Until the mid- to late 1990s, retailers typically had their own product and process specifications as well as associated verification criteria or audit schemes. As a result, a supplier often had to pass several different audits, one for each of its customers. Collaborative certification schemes, often designed for coalitions of retailers, were created to reduce the cost for certification and improve efficiency throughout the food chain. Most were designed for food generally but they are now increasingly being applied to fish and seafood products. These are arguably the most important schemes in terms of the impacts of private standards on the food industry generally – they represent comprehensive food safety management systems and are internationally significant.

In terms of food safety, most FSMSs have at their core a requirement for HACCP. Although HACCP is an internationally recognized system for risk analysis in the handling of foods, and is widely used by the seafood industry worldwide, it has become a mandatory requirement for exporting to the major markets in developed countries and emerging economies. However, HACCP is a method, and the quality of its implementation varies significantly. Several FSMSs have been developed specifically to operationalize and verify the implementation of HACCP.

Dutch HACCP: In 1996, a group of certification bodies in the Netherlands developed a standard for food safety management, “The Requirements for a HACCP based Food Safety System”. The first version of this standard was published on 15 May 1996 by the National Board of Experts HACCP, a group of experts on food safety representing all parties in the food chain in the Netherlands, and is commonly called Dutch HACCP. The foundation for the Certification of Food Safety Systems (SCV), was founded in 2004 by the National Board of Experts HACCP and the associated certification bodies. SCV acts as the legal owner of Dutch HACCP and manages this copyright with licence agreements. The latest version (Version 5, 2012) is a pure HACCP certification scheme based on the Codex Alimentarius principles. This will allow organisations to use the HACCP scheme as a starting point before later becoming certified against Food Safety System Certification (FSSC) 22000³¹, a food safety certification scheme that is also facilitated by SCV.

Food Safety System Certification 22000: The Foundation for Food Safety Certification was founded in 2004 and subsequently developed FSSC 22000. This development is supported by FoodDrinkEurope, an industry association based in Belgium. FSSC 22000 contains a complete certification scheme for food safety systems based on existing standards for certification (ISO 22000, ISO 22003 and technical specifications for sector prerequisite programmes). This scheme is intended for the

³⁰ www.naturland.de

³¹ www.fssc22000.com

audit and certification of the food safety system of organizations in the foodchain that process or manufacture:

- perishable animal products (i.e. meat, poultry, eggs, dairy and fish products);
- perishable vegetal products (i.e. fresh fruits and fresh juices, preserved fruits, fresh vegetables, preserved vegetables);
- products with long shelf life at ambient temperature (i.e. canned products, biscuits, snacks, oil, drinking water, beverages, pasta, flour, sugar, salt);
- (bio)chemical products for food manufacturing (i.e. vitamins additives and bio-cultures) but excluding technical and technological aids;
- food packaging material manufacturing.

British Retail Consortium Global Standard: In 1996, retailers in the United Kingdom of Great Britain and Northern Ireland realized that, on the issue of food safety, there were many advantages to sharing experience and developing robust systems together. The development of the BRC Global Standards was initially driven by the need to meet legislative requirements of the General Product Safety Directive of the European Union (Member Organization) and the United Kingdom Food Safety Act, that is, for retailers and brand owners to use in their “due diligence” defence should they be involved in a safety failure. It was soon seen as having significant benefits to the suppliers of product to retailers of the United Kingdom of Great Britain and Northern Ireland and subsequently, European and global retailers.

The first issue of the BRC Global Standard – Food was published in 1998. It is regarded as a benchmark for best practice in the food industry. It is a food safety and quality management protocol that includes:

- implementation of an HACCP system;
- a quality management system;
- factory environmental standards;
- product control;
- process controls;
- personnel requirements.

It has evolved into a global standard (called the Global Standard for Food Safety – Issue 6, 2012) and is used not just to assess retailers’ suppliers, but as a framework upon which many companies have based their supplier assessment programmes and the manufacture of some branded products.

Suppliers to firms under the BRC umbrella must undergo an evaluation by a BRC-certified auditor. As overseas suppliers see the benefits of accreditation to the BRC, the number of licensed certification bodies has grown. There is currently a network of about 100 accredited and BRC-recognized certification bodies around the world. The BRC has developed a database, the BRC Directory, that will allow retailers to check the status of any of the more than 13 000 suppliers in 90 countries certified to the BRC Global Standards. In the United Kingdom of Great Britain and Northern Ireland, retailers recognising BRC certification (including Tesco, Waitrose, Asda, Iceland and Sainsbury’s) account for about 90 percent of retail trade.

International Food Standard (IFS): In 2002, German food retailers from the Hauptverband des Deutschen Einzelhandels developed a common audit standard on food safety called the International Food Standard. It was designed *inter alia* to bring transparency to the supply chain. In 2003, French food retailers and wholesalers from the Fédération des entreprises du Commerce et de la Distribution joined the IFS Working Group. The IFS operates as a uniform tool to ensure food safety and to monitor the quality level of producers of retailer-branded food products. The standard can apply for all steps of the processing of foods following primary production. Rebranded as IFS Food (there are now six other IFS standards), Version 6 (is a standard for auditing quality and food safety of processes/products of food manufacturers and includes requirements about the following topics:

- Senior management responsibility;
- Quality and food safety management systems;
- Resource management;
- Production process;
- Measurements, analysis, improvements;
- Food defence.

The IFS reports association with a range of retailers and wholesalers, mainly in Europe, including: Metro Group, Edeka, Rewe Group, Aldi, Lidl, Auchan, Carrefour Group, EMC – Groupe Casino, Leclerc, Monoprix, Picard Surgelés, Provera (Cora and Supermarchés Match), Wal-Mart, Système U, COOP, CONAD and Unes. Its website notes that “Nine of the ten biggest European food retailers use the IFS as their food safety standard.”³² Registered retailers, certification bodies and certified suppliers have access to a database of IFS audit reports and certification information.

Safe Quality Food (SQF): In 1995, the Western Australia Department of Agriculture developed The Safe Quality Food Programme for the purpose of verifying the safety of food exported to other countries, particularly to the United States of America. The programme was modelled after ISO 9000 standards. In 2003, the Food Marketing Institute (FMI) based in Washington, DC, purchased the SQF programme. The FMI is a non-profit association conducting programmes in research, education, food safety, industry relations and public affairs. It has some 2 300 members, including food retailers and wholesalers, covering about three-quarters of retail sales in the United States of America. International membership includes companies from 50 countries.

In 2013, the SQF Code, Ed. 7.1 was introduced. It is an HACCP-Based Supplier Assurance code for the food industry. With this code, SQF helps make certification more attainable for smaller companies by dividing the process into three steps: from Level 1, which incorporates fundamental food safety controls appropriate for low-risk products; all the way to Level 3, indicating a comprehensive implementation of food safety and quality management systems development.

The SQF programme has been implemented by more than 5 000 companies operating in Asia-Pacific, the Near East, the United States of America, Europe and South America.³³

6.9.3 The need for harmonization

In 2013, GLOBALG.A.P., the ASC and the GAA signed a memorandum of understanding in an effort to increase efficiency and reduce the duplication in the auditing processes for farms, processing plants, hatcheries and feed mills that undertake certification by more than one of the three organization's programs.³⁴ Indeed, industry sources suggest that rivalry between schemes – particularly related to aquaculture – has created confusion in the market, with producers not sure as to which scheme, if any, to sign up to.

In terms of food safety generally (not exclusive to, but including fish and seafood products) other attempts at reducing the confusion around the proliferation of private standards, and to seek some harmonization or international norms have occurred, the first driven by an international coalition of retailers; the other in the context of the ISO.

6.9.3.1 Global Food Safety Initiative³⁵

In April 2000, chief executive officers (CEOs) from a range of international retail firms identified the need to enhance global food safety including by setting requirements for

³² www.ifs-certification.com/index.php/en/

³³ www.sqfi.com

³⁴ www.globalgap.org/uk_en/media-events/news/articles/GLOBALG.A.P.-ASC-and-GAA-Agree-to-Work-Together/

³⁵ www.mygfsi.com/

food safety schemes. They were concerned that retailers had to deal with a multitude of certificates issued against various standards in order to assess whether the suppliers of their private-label products and fresh products had carried out production in a safe manner. They noted that their suppliers were being audited many times a year, at significant cost, and with what they perceived to be little added benefit. The Global Food Safety Initiative (GFSI) was developed as an attempt to improve cost efficiency throughout the food supply chain.

The GFSI's main objective is to implement and maintain a scheme to recognize food safety management standards worldwide, including by:

- facilitating mutual recognition between standard owners;
- working towards worldwide integrity and quality in the certification of standards and the accreditation of certifying bodies.

The GFSI does not undertake any certification or accreditation activities. Instead, it encourages the use of third-party audits against benchmarked standards. The overall vision is to achieve a simple set of rules for standards, harmony between countries, and cost efficiency for suppliers by reducing the number of required audits.

A guidance document lists key requirements against which food safety management standards can be benchmarked. These requirements include three key elements:

- food safety management systems;
- good practices for agriculture, manufacturing or distribution;
- HACCP.

The application of the benchmarked standards to particular products is at the discretion of retailers and suppliers. This process will vary in different parts of the world, depending on:

- company policies;
- general regulatory requirements;
- product liability and due diligence regulations.

A number of relevant standards have been benchmarked as compliant with the GFSI including (as of August 2013):³⁶

- Global Aquaculture Alliance Seafood Processing Standard
- GLOBALG.A.P. Integrated Farm Assurance Scheme and Produce Safety
- Food Safety System Certification 22000
- SQF Code 7th Edition Level 2
- BRC Global Standard for Food Safety Issue 6
- IFS Food Version 6

The GFSI board, made up of representatives from the largest retail and wholesale food companies in the world, is the main governing body. It is responsible for policy-making and overall decisions, and it is supported by a task force, which acts as a consultation body. Overall, the GFSI accounts for more than 70 percent of food retail sales worldwide.

The GFSI is an important development in that it is an attempt to reduce the transaction costs associated with retailers and their suppliers having to apply a multitude of different standards. Suppliers to European retailers report needing BRC certification for the market in the United Kingdom of Great Britain and Northern Ireland and IFS certification for the German and French markets. In theory, having a standard benchmarked against the GFSI should mean that there is some form of mutual recognition or equivalence.

All the schemes benchmarked to the GFSI require traceability systems and monitoring as well as auditing in line with Codex and HACCP. In practice, differences remain in terms of the specific requirements of schemes and their related certification and audit processes. Indeed in a survey conducted by the Organisation for Economic

³⁶ www.mygfsi.com/about-gfsi/gfsi-recognised-schemes.html

Co-operation and Development (OECD, 2006), retailers that were members of the GFSI reported that they not only used GFSI benchmarked standards, but often a combination of them. Moreover, they also often add on standards specific to the firm. This is especially the case with owners of private-label and brand-name products. Many retailers remain members of several schemes. Carrefour, for example, is a member of the GFSI, the IFS, and of the FMI (which owns the SQF). In the United Kingdom of Great Britain and Northern Ireland, Tesco is a member of the SQF, BRC and GFSI. Work has also been undertaken by the GFSI on differences and similarities with ISO 22000 (described below).

The retailer members have all agreed to reduce duplication in supply chains through the common acceptance of any of the GFSI benchmarked schemes. The impacts on suppliers will need to be monitored. Whether the GFSI has reduced the proliferation of private standards remains to be demonstrated, but it has increased awareness of global food safety issues and facilitated cooperation between international retailers.

6.9.3.2 *International Organization for Standardization – ISO 22000*

In addition to the adoption of private standards, many food companies and retailers have also adopted international voluntary standards developed in the context of the ISO. The ISO is a network of national standards bodies, based in Geneva, Switzerland. It is an NGO that is the product of collaboration between public and private sector bodies. Its members include national standardization bodies as well as industry associations. Despite this public-private mix, the WTO recognizes the ISO as providing internationally recognized standards, which allows some assurance of safety and quality across national borders.

In the late 1980s, the ISO developed the ISO 9000 series for quality management in all sectors. Although ISO 9000 helped food companies to improve the organizational and operational aspects of quality management, it lacked food safety specifics, especially reference to HACCP requirements. Subsequently, ISO 22000 was developed in 2005, building on previous food-safety-related standards, as an attempt to establish one internationally recognized standard for food safety management systems. However, to date, it sits alongside the range of other private and public schemes. The ISO 22000 family of standards contains a number of standards each focusing on different aspects of food safety management.

- ISO 22000:2005 contains the overall guidelines for food safety management.
- ISO/TS 22004:2005 contains guidelines for applying ISO 22000
- ISO 22005:2007 focuses on traceability in the feed and food chain
- ISO/TS 22002-1:2009 contains specific prerequisites for food manufacturing
- ISO/TS 22002-3:2011 contains specific prerequisites for farming
- ISO/TS 22003:2007 provides guidelines for audit and certification bodies

There has been some collaboration between the ISO and the GFSI. For example, the ISO participates in the GFSI Technical Committee. A comparison conducted by the GFSI of the GFSI Guidance Document and ISO 22000³⁷ showed strong similarities. However, different approaches to accreditation and differences in ownership – the retailer-driven GFSI versus the diverse public-private ISO 22000 membership – were cited as the stumbling blocks to formal recognition by the GFSI of ISO 22000. It was thought that the retailer-driven GFSI benchmarked schemes had a “specific reactivity” and could implement changes agreed in the GFSI, whereas the decision-making structures of the ISO were thought to be less conducive to “timely and efficient” adjustments in the light of changes in market conditions and demand.

³⁷ “What is ISO 22000?” www.ciesnet.com.

More recently, the ISO Technical Group 234 on fisheries and aquaculture has developed standards for the traceability of both captured (ISO 12875:2011) and farmed (ISO 12877:2011) finfish products.

6.9.3.3 Global Sustainable Seafood Initiative

In February 2013, seventeen leading companies of the seafood industry and the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH on behalf of the German Federal Ministry for Economic Cooperation and Development (BMZ) entered a strategic alliance called the Global Sustainable Seafood Initiative (GSSI). The mission of GSSI is to deliver a common, consistent and global benchmarking tool for seafood certification and labelling programs to ensure confidence in the supply and promotion of sustainable seafood to consumers worldwide, as well as promote improvement in the certification and labelling programmes.

In the first four months the GSSI formalized its mission and objectives, increased its partnership from 17 to 30 companies from around the globe, organized stakeholder workshops to collect feedback and recommendations at major seafood shows and have created three expert working groups to advise the GSSI Steering Board on the development of a benchmark framework (a) to evaluate aquaculture certification standards; (b) to evaluate fisheries certification standards; and (c) to evaluate all other aspects (e.g. governance, standard setting procedures, certification, accreditation, chain of custody) of a certification programme, as well as describe the benchmark process.

The GSSI is in its infancy and the goal is to complete the benchmarking process over a three year period.

6.9.4 Calls for international guidance

The above descriptions attest to the multitude of different food safety management systems and related private standards that have emerged in the past decade and a half, and which are increasingly being applied to fish and seafood. Despite attempts at harmonization, there is little evidence to date to suggest that retailers are prepared to give up their own mix of specifications and requirements for certification. Instead, it appears that global schemes sit over national collaborative schemes, which individual retailers sign up to and then add on their own individual product and process specifications (related to safety and quality as well as other aspects of their corporate social responsibility policies). This is perhaps the clearest evidence that private standards are not only designed to provide guarantees against food safety failures, they are also tools for differentiating retailers and their products.

The work of the GFSI and the development of the ISO 22000 family of standards, and the specific cooperation between GLOBALG.A.P., the ASC and the GAA in aquaculture, are indicators of the need for some harmonization of private standards. International organizations have been asked to play a role in this context. Discussions on private standards generally have been held in the context of the WTO. The OECD has carried out a number of studies on private standards, albeit concentrating on agricultural products and excluding fish and seafood. (OECD, 2006). FAO has been asked by its Members, in the context of COFI to help clarify and resolve some of the challenges related to private standards as they apply to fish and seafood. Discussions have been had in the context of two COFI subcommittees: on aquaculture, and on fish trade.

6.9.5 Pressure on developing countries to meet private standards

As noted in earlier chapters, developing countries represent about half of world exports of fish and fishery products by value and about 60 percent in terms of quantity (FAO, 2009a). Developing countries have expressed concerns, for example in the context of

COFI and the WTO, that private standards could pose a barrier to their access to international markets.

Research on the implications of private standards and retailer procurement strategies on developing country producers and processors is limited. However, it appears that, with the exception of aquacultured shrimp, developing countries have so far had relatively little exposure to the pressure to comply with private standards. This is due to three key factors:

- They supply proportionately smaller volumes into markets where private standards are most prevalent.
- They supply non-processed, or minimally processed, fish and seafood while private standards apply mainly to processed value-added products for brands or private labels.
- They tend to operate in supply chains with low levels of integration and, therefore, limited direct interface with retailers and private standards schemes.

The markets that are most demanding in terms of private standards are the markets where imports from developing countries are lowest. For example, the percentage of European imports from developing countries that end up in Germany and the United Kingdom of Great Britain and Northern Ireland, where private labels and private standards are more dominant, are relatively low. These markets tend to prefer North Atlantic and North Pacific species to tropical species from developing countries (again, with the notable exception of shrimp, catfish and species typically sold as canned products: tuna, sardines, etc.).

Furthermore, an FAO study of developing country products on sale in supermarkets in France and Italy found that “One of the striking features is the absence of prepared seafood in the developing country range.” (FAO, 2008b). The study estimated that processed products from developing countries accounted for less than 10 percent of retail sales of processed fish and seafood in those markets. Fish and seafood from developing countries tends to be imported as frozen whole fish or fillets. These products demand few requirements above those mandated by public regulation. A large proportion of value-added seafood products on sale in Europe, with the exception of canned fish (tuna, anchovies, mackerel and sardines), has been processed in factories located in Europe (or some other third country). This is where the responsibility for complying with private standards would fall.

As noted above, differences in supply chain structures will have most impact and result in differences in the implementation of food safety and quality control systems and exposure to pressure to comply with private standards. Three types of supply chains are discussed below in relation to developing countries.

Vertically integrated supply chains: “In the vertically integrated supply chain, the chain activities of fish farming/harvesting, processing and transportation to the European wholesaler/retailer are fully under the control of one transnational company (in most cases of Western origin)” (World Bank, 2005). Large retailers or processors typically source fishery products from developing countries through “wholly or partly owned processing facilities in these countries or through contracts with independent firms in the developing countries” (FAO, 2008b). Under this scenario, information about safety and product specifications flows down to producers, sometimes via representatives of the company based in the producer country. Producers are therefore linked into the production process and are supported in their activities, including with compliance to private safety and quality standards.

This would be the minority scenario for most developing country producers and processors. While acknowledging the limited evidence of its own inquiry, an FAO study concluded that: “developing countries have yet to exploit the benefits from value addition gains associated with product certification”. (FAO, 2008b).

Collaborative supply chain: A second type of supply chain is characterized by larger producers or groups of producers that work with exporters that in turn, via their relationships with importers, translate market specifications back down to those producers. This can apply to both the wild-catch sector and to aquaculture. In terms of developing countries, “most European importers who source fish from a particular country or from selected traders have established local offices in the developing countries to co-ordinate activities in the supply chain (processing, transportation, quality control, export papers)”. (World Bank, 2005). The importer advises the chain actors as to food safety and quality requirements, both public and private. This type of chain was found to be operating for Nile perch (from Lake Victoria in East Africa) and some farmed tilapia. Under this scenario, importers are the link between the source and the market, making the complexity and evolving nature of the market requirements understood by producers. It is this intermediary that experiences the most pressure to respond to private standards, including by seeking additional information about methods implemented at earlier stages of the supply chain.

Importer-driven or fragmented supply chain: Where there is a more fragmented supply chain, categorized by a range of small-scale suppliers, there are less-direct relationships by which information about food safety and quality requirements can be passed on to producers. Those producers typically sell into open commodity markets via an intermediary buyer/exporter. At the production end, there is little information about the specifications required at the import end. Under this scenario, there is a reliance on product testing at the point of importation, as safety management systems further down the chain cannot be guaranteed. Most of the exports from developing countries are traded in this type of supply chain. As the FAO (2007) study in the Asia-Pacific area explained: “For small-scale farmers, establishing a direct link with the market would be in most cases almost impossible. Farming systems in the Asia-Pacific region are in fact dominated by networks of traders which are making quality assurance and traceability huge challenges for all stakeholders ... for small-scale producers to have access to and benefit from a certification schemes they would have to be part of more direct supply chains.”

In conclusion, in terms of the three chains, only producers in the first and the second would have any interface with private standards, the first directly, and the second indirectly whereby standards are translated via close exporter–importer relationships. However, most of the fish from developing countries is traded via the latter type of supply chain, that is: “in commodity trade arrangements [where] little is traded in more secure supply contracts or conducted as a result of transfer trading between companies that relate to each other through shared equity”.³⁸ Therefore, it seems that, to date, developing country producers, and most processors, have experienced minimal pressure to comply with and be certified to a private standards scheme. However, a negative aspect of this is that their limited interface with private standards reflects their inability to engage with such schemes. The result is that they are missing out on the opportunities such schemes might offer in terms of the potential to produce more value-added products and to access lucrative segments of developed country markets.

6.9.6 Conclusion and future areas for attention

The impact of private safety and quality standards is likely to increase as supermarket chains increasingly dominate the distribution of fish and seafood products, and as their procurement policies move away from open markets towards contractual supply relationships. These supply relationships are increasingly defined by private standards with detailed product and process specifications. As large European retailers (the vast majority of leading retail transnationals, with the exception of Wal-Mart, are Western

³⁸ Alastair MacFarlane, in OECD/FAO (2007, p. 26).

European) become increasingly globalized, their buying strategies will influence retail markets in East Asia, Africa, Eastern Europe and Latin America.

While there are many opinions on the impacts of private standards on global food governance and international trade generally, there remains a dearth of empirical evidence. In terms of international trade in and marketing of fish and seafood, the gaps in evidence are even more pronounced. Some key questions remain.

6.9.6.1 *Are private standards adding value to food safety governance?*

Whether or not private standards are adding value to food safety governance is arguably in the eye of the beholder. For retailers seeking quality assurance, robust risk management and clear lines of traceability, then the answer is undoubtedly “yes”. They do address additional quality requirements, document the implementation of good practices and provide a separate level of assurance for liability purposes. However, in terms of bottom-line food safety and consumer protection, the answer is probably “no”. Most private FSMSs are based on mandatory regulation with additional specifications related mainly to quality aspects and the aforementioned risk and traceability assurances. While there has been no systematic comparison of the private sanitary requirements of individual firms with those encapsulated in public regulation, industry sources supplying to those firms suggest that key safety criteria (such as “use by” dates, and acceptable levels of additives or contaminants) are not more stringent than those required by public authorities. In any case, both public and private standards are typically based on Codex and its guidance for HACCP. Despite some misconceptions that private standards schemes encapsulate lower levels of “tolerance” – or zero tolerance (Box 1) – there is no evidence that they are stricter in terms of food-borne hazards, or that they have reduced the incidence of food scares, or that they result in safer food.

Comparing private standards with international public standards, such as Codex, as envisaged in WTO discussions, would also be useful. However, it should be noted that while these standards are developed in an international context and by consensual agreement, the monitoring and verification aspects of compliance are left to individual national authorities. As noted above, large-scale retailers requiring certification to private standards express a lack of confidence (whether justified or not) in the “competence” of some competent authorities.

What is definitely not adding value to global food safety governance is the growing proliferation of private standards and certification schemes. It has led to confusion and could undermine confidence in standards overall. Various stakeholders at different levels of the supply chain have expressed concerns about the number and varying quality of schemes. Producers and processors are unsure as to what scheme to seek certification against, and even retailers and large brand owners have doubts about which FSMSs are most robust. Signing up to a rainbow of schemes – for example, an FSMS, a specific aquaculture certification, and some environmental standard, or some combination of these – creates inefficiencies and unnecessary costs. A plethora of labels on one product is likely to result in confusion rather than customer confidence.

6.9.6.2 *Evaluating the relative quality of private standards certification schemes*

As in the area of ecolabels, industry sources have highlighted the need for a benchmark against which to judge the quality and credence of the various certification schemes. The aforementioned GFSI has a mechanism for this in terms of FSMSs. A gap exists for aquaculture certification schemes, though the recently established (in 2013) Global Sustainable Seafood Initiative (GSSI) is addressing this, both for capture fisheries and for aquaculture certification. The FAO Guidelines on Aquaculture Certification provide minimum substantive requirements against which aquaculture certification schemes can be assessed, and these guidelines form the basis for the GSSI benchmarking for aquaculture.

BOX 1 Zero tolerance

Zero tolerance is a powerful term, with the intended connotation of the complete absence of the hazard¹ or inappropriate behavior at issue, and it is popularly perceived as assurance of protection against--or at least official intolerance of--that hazard or behavior. The term zero tolerance is commonly used in the media in many contexts, including food safety. For example, zero tolerance has been used to comment about drug-law enforcement, drug-testing policies in sports, crime, and security violations. Businesses frequently highlight their zero tolerance of offensive behavior (for example, zero tolerance for hate messages in chat rooms and message boards) or consumer protection.

In food safety, the term zero tolerance often resonates well with the public which is seeking assurance of the safety of the products it consumes. Consequently, food safety regulators often confront the notion that they should have “zero tolerance” policy for anything that is deemed to pose a risk² to public health or safety, including in reference to a pathogen or environmental contaminant to indicate that whenever a particular problem is found, strict regulatory action will be taken.

But, zero tolerance in food safety does not always mean zero risk or total absence of a contaminant in a food. For example, there can be no zero risk (total absence) for some contaminants such as mercury in fish and seafood, because mercury is a natural contaminant of the aquatic environment which naturally finds its way through the aquatic food chain and bio-accumulation into some seafood. Likewise, certain bacteria such as *Vibrio parahaemolyticus* are part of the normal flora of the aquatic environment, but represent a hazard only at high concentrations. In this case, the regulatory zero tolerance policy will aim to ensure the presence of the contaminant only at levels far below the hazard level, to ensure no health risks to consumers.

Scientists are often dismayed by the use of this term because they recognize the inability to ensure, in many situations, the complete absence of certain pathogens and contaminants from the food supply and the limitations of feasible sampling plans to check for their total absence (see box 12). But, scientists do recognize that a preference for zero “is influenced by the wish to emphasize that absence of the hazard is the desired objective (although it cannot be always guaranteed) and by the knowledge that once pathogens or contaminants are found, the finding cannot be ignored”. The various uses of and limits of this term, therefore, must be properly analyzed and understood.

¹ In food safety, hazard is defined as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect (Codex alimentarius)

² A risk is defined as a function of the probability of an adverse health effect and the severity of the effect, consequential to a hazard(s) in food.

Source: Washington and Ababouch (2011).

While the FAO Members have agreed to guidelines for aquaculture certification, there is less agreement – and no clear mandate – as to whether FAO should assess any private scheme against those criteria.

6.9.6.3 Do private standards conflict with, complement or duplicate public regulation?

Here again, because there has been no systematic comparison of private standards with public regulation, there is no concrete evidence to assess the relationship between public and private standards. Several areas are especially pertinent.

Food safety: As noted above, private standards are typically based on mandatory regulation and, therefore, are not likely to demand more in terms of acceptable levels

of biological or chemical contaminants, or more stringent “use by” dates, etc. Hence, they are unlikely to conflict with public food safety regulation. Duplication is more likely to be an issue, if not in relation to the content of requirements, then in methods of compliance and verification (including multilevel documentation).

Concerns about having to comply with a variety of standards need to be addressed. Those concerns are likely to mirror concerns about the relative lack of harmonization of public regulation, including the lack of harmonization between the safety and quality requirements of public authorities in various export markets. Some harmonization and mutual recognition of public regulatory frameworks for food safety would go a long way towards reducing the current complexity in global food safety governance and would ease international trade. Improved dialogue between the public and private sectors at the international level, with the aim of reducing the complexity of food safety governance overall, would be useful (the GFSI and ISO are observers at Codex and vice versa, and the dialogue between the ISO and GFSI might act as a harbinger).

There is little evidence to suggest that compliance with private standards might facilitate the implementation of public standards. Indeed, the inverse is a more likely scenario. Compliance with public standards provides a baseline, and is therefore essential for meeting the requirements included in private standards schemes.

Do demands from buyers for suppliers to be certified and the certification process itself incentivize better food safety management, or are operators that achieve certification mainly those that already run effective food safety management systems? A further key question for policy-makers, especially in the context of an apparent shift in responsibilities from the public to private sector for food safety management is: Are profit-maximizing private sector firms the best agents for incentivizing better food safety management throughout the supply chain?

Traceability: The traceability requirements of private standards schemes – often requiring full traceability from farm/boat to fork – are likely to be as, if not more robust, than most public requirements. The traceability requirements of the European Union (Member Organization) are arguably the most stringent in terms of public regulatory requirements, based on the principle of “one step backwards, one step forwards”,³⁹ and requiring all aspects of the supply chain⁴⁰ to be approved by the competent authority approved by the European Commission. However, as noted above, private standards schemes require traceability requirements to be verified by private-sector certification companies, because public audit reports are not readily available to buyers. Assisting with capacity building in countries with weak administrative systems would arguably be a more effective strategy than imposing a parallel private system to compensate for perceived or real administrative shortcomings. Moreover, a company certified to a private standards scheme will still not have access to certain markets, such as the European Union (Member Organization), if the competent authority of the country in which it operates has not been approved by public authorities in key import markets.

Audit and documentation – duplication and complexity: It is in the area of audit and verification and the related documentation required where duplication between public and private requirements is perhaps most evident. Separate sets of compliance documents relating to public and private certification (or even several public and several private certifications) amount to heavy compliance costs. Such costs are especially burdensome where there is a prescriptive rather than an outcome-based approach to compliance. It has been argued that while the public-sector trajectory is towards more outcome-oriented systems (defining outcomes or ALOPs, and allowing operators the flexibility to choose how to achieve them), private standards schemes

³⁹ See: Exporting Seafood to the European Union, International Trade Centre, Bulletin No. 84/2008/Rev.1

⁴⁰ Vessels, landing sites, transporters, processors, etc. for capture fisheries and feed producers, hatcheries, farms, transporters, processors, etc. for aquaculture products.

remain wedded to a substantive checklist approach including precise product and process requirements. There is a need to promote more outcome- or performance-based compliance management and verification. Producing two (or more) compliance documents according to who is conducting an audit is not only "... a waste of resources, it diminishes the value of true compliance, as it is seen as a paper exercise",⁴¹ rather than as a tool for continuous management and quality improvement.

6.9.6.4 Areas for attention

The above sections have highlighted the dearth of empirical evidence and the need for further research and some action in the following areas:

- **Comparing public with private standards for safety and quality management.** Comparisons of public with private food safety management requirements are needed to determine where there are synergies to be exploited, efficiencies to be gained, and duplication to be avoided. Moreover, what role can and should the public sector take in regulating the activities of private-sector standards schemes?
- **Private safety/quality standards and impacts on international trade.** There is a need for more evidence and analysis on the impacts of private standards on international trade based on concrete country evidence. Do they really act as non-tariff barriers to trade, generally, and specifically in relation to fish and seafood?
- **Assessment tools and methodological advancement.** There is a need for some guidelines or assessment criteria so that industry players can judge the quality of private standards schemes to assess which certification schemes carry most value and have most credence in the market. The GFSI provides a mechanism for benchmarking FSMSs and food safety generally, which covers fish processing activities whether from wild capture or aquaculture sources. The forthcoming FAO aquaculture guidelines will provide minimum criteria for aquaculture certification schemes.
- **Harmonization and mutual recognition – public and private.** There is a need for further harmonization of government food safety regulations. This is gradually being implemented by the relevant Codex Committees and by the OIE. The GFSI goal of "once certified, accepted everywhere" is a step towards harmonization of private FSMSs. The FAO aquaculture guidelines provide the basis for mutual recognition of certification schemes specific to aquaculture and are being used by the GSSI for this purpose. The interface between public and private harmonization efforts could be explored further. The key question is which overall global food safety governance framework will best serve consumer protection and public health, as well as industry needs for traceability and risk management, while also promoting efficiencies for the various stakeholders in the supply chain. Some sort of roadmap with desired outcomes and interim deliverables would need to be developed with both public- and private-sector participation. This would facilitate trade and decrease the current complexities in global food safety governance.
- **Support to developing countries.** Support to developing countries would probably be best in the form of assistance to improve the infrastructure (physical, regulatory and institutional) that is a prerequisite for compliance with both public and private food safety and quality standards. This might involve some supply chain development. The transfer of information, technology and expertise from integrated supply chain actors to other parts of the industry might help fisheries stakeholders move beyond

⁴¹ Francisco Blaha, FAO, personal communication, 11 February 2009.

“entry-level commodity trading relationships with international markets” to take advantage of opportunities for more value-addition and, subsequently, improve access to more lucrative markets or market segments in importing countries. Documenting success stories and sharing these with industry stakeholders in other developing countries would be valuable. In particular, sharing examples of how small-scale fisheries and aquaculture operations have organized to achieve export success (including through group certification) would be useful (Box 2).

BOX 2

India – clustering fish farms to improve production and market access

Ninety five percent of Indian aquaculture shrimp and prawns are exported. The demands of international markets, including for certification, have been problematic for Indian farmers. As 90 percent of them operate ponds that are smaller than two hectares, traceability and meeting certification requirements and costs is especially difficult. To counter some of these problems, the aquaculture industry is now regulated by the Coastal Aquaculture Authority Act, which includes codes of practice for aquaculture operators and registration of farms, hatcheries and processors.

In 2006, the Marine Products Export Promotion Authority (MPEDA) of India, which operates under the auspices of the Ministry of Commerce and Trade, created the National Centre for Sustainable Aquaculture (NACSA), headquartered in Kakinada, Andhra Pradesh, with the mission to organize small-scale fish farmers into societies that can collectively benefit from the NACSA’s technical support and advice to address production and market access issues. The aim is to promote sustainable small-scale aquaculture through empowerment of farmers to access credit, quality seeds, feeds and other inputs and to implement better management and good aquaculture practices to reduce fish diseases, improve product quality and access international markets, including through certification.

The farmers’ societies have clear organization with strict conditions for membership and elected board members. In addition to training and awareness improvement programmes for society farmers, the NACSA technical staff monitor inputs (seed, feed) to ensure the use of disease- and residue-free inputs and proper traceability. The NACSA is developing a digitalized database supported by GIS for all society farms. Ponds will be identified by a nine-digit code, with each society maintaining a complete record from stocking to harvest, including traceable seed and feed.

In 2009, the NACSA reported more than 7 000 farmers organized into 250 societies. The NACSA aims to organize 75 000 farmers into 1 500 societies by the end of 2012. The experience since 2007 has demonstrated major benefits for farmers in terms of access to microcredit, better bargaining position for inputs and final product prices, as well a better integration of the sector (hatchery–society–processor/exporter).

Source: Washington and Ababouch (2011).

6.10 SAFETY AND QUALITY MANAGEMENT IN AQUACULTURE (LAHSEN ABABOUC, ROHANA SUBASINGHE AND IDDYA KARUNASAGAR)

6.10.1 Introduction

Food safety hazards associated with aquaculture products will differ depending upon the farmed species, the region, the habitat, the method of production, management practices and environmental conditions. The origins of such food safety concerns are diverse, ranging from inappropriate aquaculture practices to environmental pollution and cultural habits of food preparation and consumption.

Chapter 3 describes the main food safety hazards and their control methods as they apply to products of aquaculture. These hazards can be caused by parasites (nematodes or round worms, cestodes or tapeworms and trematodes or flukes), bacteria (*Vibrio* spp., *Aeromonas* and *Plesiomonas* spp., *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, etc.) or chemical contaminants (residues of pesticides, veterinary drugs, heavy metals or additives).

Regarding quality, aquaculture products can develop quality defects similarly to the corresponding wild fish species, although the possibility of better control in aquaculture production can minimize these defects, particularly flavour quality. Moreover, during harvesting and transport of live fish, by reducing stress, fish physical damage and bruising will be minimized.

Similarly to wild capture fisheries, the assurance of fish and seafood safety and quality in aquaculture requires the adoption and implementation of GAPs as prerequisites for the implementation of the HACCP system. Chapter 6 of the Codex International Recommended Code of Practice covers aquaculture but only intensive or semi-intensive aquaculture systems that use higher stocking densities, stock from hatcheries, use mainly formulated feeds and may utilize medication and vaccines. It does not cover the extensive fish farming systems that prevail in many developing countries or integrated livestock and fish culture systems.

The following applies to all kinds of aquaculture production systems. It covers all aquatic animals, except mammalian species, aquatic reptiles and amphibians for direct human consumption, but excludes bivalve molluscs. The main stages of aquaculture production covered are site selection, growing water quality, source of fry and fingerlings, feeding, growing, harvesting and transport.

Prior to the implementation of GAPs and HACCP, aquaculture establishments should operate in a responsible way such that they comply with the recommendations of the Code of Conduct for Responsible Fisheries (FAO, 1995) in order to minimize any adverse impact on human health and the environment, including any potential ecological impact.

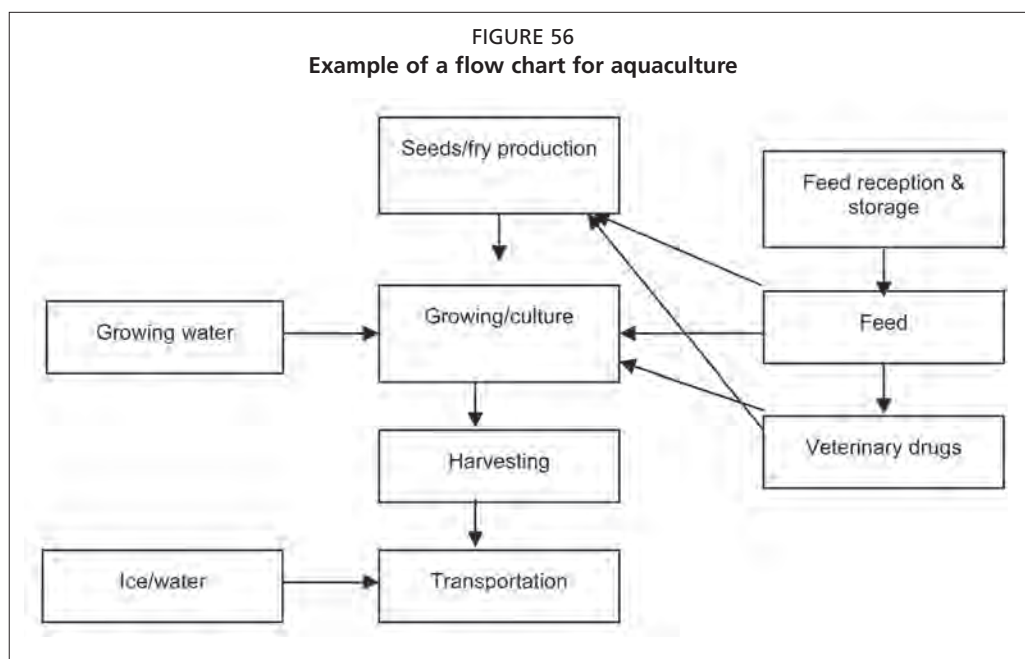
Fish farms should operate effective fish health and welfare management. Fry and fingerlings should be disease-free and should comply with the OIE Codes of Practice (International Aquatic Animal Health Code, sixth edition, 2003). Growing fish should be monitored for disease. When using chemicals at fish farms, special care should be exercised so that these substances are not released into the surrounding environment.

While the implementation of HACCP-based food safety assurance programmes is well advanced in the fish-processing sector, the application of such programmes fish farms is in its infancy. One possible explanation is linked to the fact that major food safety regulatory agencies, such as the FDA or the European Commission Food and Veterinary Office have not made the application of HACCP in aquaculture mandatory, although Codex supports its application.

While many recognize that the application of HACCP may be difficult at the “farm level”, a number of experts consider the application of HACCP in aquaculture feasible, cost-effective, and an effective complement to biosecurity measures taken to prevent fish diseases, especially in well-organized farms of suitable size. Currently, in several countries around the world, an increasing number of aquaculture farms are applying HACCP-based concepts to control food safety issues. The challenge for small-scale farmers is being tackled in many countries, such as India, Thailand, Viet Nam and Indonesia, by organizing the farmers into clusters or self-help groups, whereby they can reach a size suitable for the application of GAPs and HACCP, and benefit from the certification thereof.

6.10.2 Good aquaculture practice

Recognizing that aquaculture systems may differ considerably depending on the species cultivated, the Codex code of practice offers the general flow chart of Figure 56, reproduced here for illustrative purposes. This chart represents the common steps in aquaculture production, namely: (1) site selection; (2) growing water supply; (3) source of fry and fingerlings; (4) feed supply; (5) veterinary drugs; (6) growing; (7) harvesting; (8) holding and transportation; and (9) storage and transport of live fish.



To prevent food safety and quality problems originating at the farm level, the following good practices are recommended at each step.

6.10.2.1 Site selection

Fish farms should be located in areas where the risk of contamination by biological, chemical or physical food safety hazards is minimal and where sources of pollution can be controlled. All potential sources of contamination from the environment should be considered. In particular, fish farming should not be carried out in areas where the presence of potentially harmful substances would lead to unacceptable levels of such substances in fish.

- Food safety hazards can arise from the location of the fish farm as a result of its surroundings, through the water supply, direct contact with animals, or airborne contamination (i.e. chemical sprays). Nearby agricultural lands that use pesticides and heavy fertilization on a regular basis could be a potential source of contamination.
- Livestock (cattle, ducks, pigs, chickens, etc.) farms or sewage effluents can be a serious source of contamination by pathogens (i.e. parasites such as *Opisthorchis*, or pathogenic bacteria such as *Salmonella*). Fish farms need to be located away from any of these activities to eliminate the risk of contamination.
- Soil for the construction of earthen ponds should not contain toxic chemicals and other substances that can contaminate the fish.
- All the sites should be operated so as not to cause an adverse impact on human health from the consumption of the fish in the farm.

6.10.2.2 Growing water supply

The water in which fish is raised should be suitable for the production of food that is safe for human consumption. Fish farms should not be sited where there is a risk of contamination of the water in which fish are reared by chemical and biological hazards. Water sources should be protected from contamination by wild and domestic animals, effluents and runoffs.

- Freshwater fish cultured in certain parts of the world where food-borne trematodiasis (*Clonorchis*, *Opisthorchis*) is endemic may harbour the infective stages of the parasites.
- Aquatic birds are known to harbour pathogenic parasites (*Haplorchis*, *Diphyllobothrium* and others) and pathogenic bacteria (*Salmonella*, *Vibrio cholerae* and others) and are possible sources of these organisms on fish farms. Wild (lizards, snakes, turtles, rats) and domestic (cattle, pigs, chickens, ducks, cats and dogs) animals are other potential sources of such biological contamination.
- The use of wastewater for fish farming or the practice of fertilizing ponds with human waste and untreated animal manure may result in products that harbour pathogenic bacteria and parasites. Manure from animal production facilities can be contaminated with drugs added to animal feed for the prevention of disease. These substances can potentially pass from the manure to fish and cause food safety concerns. The WHO guidelines for the safe use of wastewater in aquaculture should be followed (WHO, 2006a).
- Fish farms should be designed and constructed to ensure control of hazards and prevention of water contamination. Water inlets and outlets to ponds should be screened to prevent the entrance of unwanted species.
- Water quality should be monitored regularly to prevent fish contamination during production.

6.10.2.3 Source of fry and fingerlings

Sources of post-larvae, fries and fingerlings should be controlled in order to avoid the carryover of potential hazards into the growing stocks.

- In endemic fish-borne parasite areas, the source of fries and fingerlings should ensure that seeds are free from parasitic infection. Contaminated sources are common in endemic trematodiasis areas.

Feed supply

Feeds can transmit harmful agents directly or by attracting pests.

- Feeds used in aquaculture production should comply with the Codex Code of Practice on Good Animal Feeding (CAC/RCP 54-2004). Feed ingredients should not contain unsafe levels of pesticides, chemical contaminants, microbial toxins, or other adulterated substances.
- Ingredients should meet acceptable levels of pathogens, mycotoxins, herbicides, pesticides and other contaminants that may give rise to human health hazards.
- Feed should contain only such additives, growth-promoting substances, fish-flesh colouring agents; anti-oxidizing agents, caking agents or veterinary drugs that are permitted for fish by the official agency having jurisdiction.
- Industrially produced feeds and feed ingredients should be properly labelled. Their composition must fit the declaration on the label.
- Medicated feeds should be clearly identified in the package and stored separately, in order to avoid errors.
- Products should be registered with the relevant national authority as appropriate.

- Storage and transport conditions should conform to the specifications on the label.
- Feed should not be used after the expiry of shelf-life.
- Dry fish feeds should be stored in cool and protected dry areas to prevent contamination, mould growth and spoilage. Moist feed or feed ingredients should be properly refrigerated and reach the fish farm in an adequate state of freshness.
- Fish silage, low-value fish and offal from fish, if used, and where necessary, should be properly cooked or treated to eliminate potential hazards to human health.
- Product tracing of all feed and feed ingredients should be ensured by proper record-keeping.

6.10.2.5 *Veterinary drugs*

All veterinary drugs for use in fish farming should comply with national regulations and international guidelines, in accordance with the Recommended International Code of Practice for Control of the Use of Veterinary Drugs (CAC/RCP 38-1993) and the Codex Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drug Residues in Foods (CAC/GL 16-1993 – under revision – the revised text will also supersede CAC/RCP 38-1993). Products should be registered with the appropriate national authority.

- Control of diseases with drugs should be carried out only on the basis of an accurate diagnosis. Products should only be prescribed or distributed by personnel authorized under national regulations.
- Veterinary drugs or medicated feeds should be used according to manufacturer's instructions, with particular attention to withdrawal periods.
- Prior to administering veterinary drugs, a system should be in place to monitor the application of the drug and respect of the withdrawal time for the batch of treated fish.
- Storage and transport conditions should conform to the specifications on the label.
- Records should be maintained when veterinary drugs are used.

6.10.2.6 *Growing*

The growing phase includes various activities that can significantly affect the safety and quality of farmed fish. There is a need to control the growing water quality, the design and cleaning of equipment and holding facilities, the maintenance of pond grounds, the workers hygienic practices and pests.

- Equipment such as cages and nets should be designed and constructed to ensure minimum physical damage of the fish during the growing stage.
- Equipment and holding facilities should be easy to clean and to disinfect and should be cleaned and disinfected regularly and as appropriate.
- Farm grounds should be well maintained to reduce or eliminate food safety hazards. By keeping plants around the ponds (i.e. mangroves), farmers can reduce erosion that carries chemical and biological contamination to the pond. At the same time, it is necessary to keep the grounds clean of high, excessive weeds, and trash and debris that can attract pests.
- Good hygiene practices in the pond area should be applied to minimize faecal contamination of pond water. A major concern is the contamination by pathogenic bacteria or parasites from waste materials or faecal matter from mammals, animal or humans. Farm personnel should not be allowed to defecate in the estuary, ponds, on the groundwater near the estuary or ponds, or any place from where rain can wash the faeces into the estuary or ponds.

In the absence of toilets, defecation can be done in designated receptacles (i.e. plastic buckets), latrines or field toilets that are subsequently treated with disinfectant (lime or chlorine) and whose waste is disposed of in a sanitary manner. These field sanitary facilities should be located away from ponds or water sources, and should be regularly maintained to prevent potential leakage into ponds or source water. Another way to dispose of disinfected human waste is to burn the excrement in a designated burning receptacle.

- Fish farms should institute a pest control programme. Rodents (rat, mice, nutrias, etc.), birds (ducks, cormorants, etc.) and other wild animals (e.g. snakes, turtles and lizards) can be a source of microbial or parasite contamination. Rodent control, using trap and bait systems, around storage areas is essential. Birds can carry microbial and/or parasite concerns to the pond and cause an economic problem by preying on fish. Traditionally, birds have been controlled by placing nets or wires over small ponds or by using loud noises for larger ponds. Domestic animals such as dogs, cats and pigs should receive adequate de-worming treatment in endemic parasitic areas such as those affected by food-borne trematodiasis.
- Good water quality should be maintained by using stocking and feeding rates that do not exceed the carrying capacity of the culture system. Stocking densities should be based on culture techniques, fish species, size and age, carrying capacity of the fish farm, anticipated survival and desired size at harvesting.
- Diseased fish should be quarantined when necessary and appropriate, and dead fish should be disposed of immediately in a sanitary manner.

6.10.2.7 *Harvesting*

Appropriate harvesting techniques should be applied to minimize spoilage, physical damage and stress (live fish).

- Harvesting should be rapid so that fish are not exposed unduly to high temperatures. In tropical areas, harvesting should be done at night at a time when the temperature is lowest.
- Soon after harvest, fish should be washed using clean seawater or freshwater under suitable pressure to remove excessive mud and weed.
- Soon after harvest, fish should be iced or immersed in ice slurry to reduce their temperature to about 0 °C.
- Equipment and utensils such as nets, bags, pumps, baskets, tubs, bins and boxes, should be designed and constructed to ensure minimum physical damage to the fish during harvesting. All equipment and utensils used during harvesting should be easy to clean and to disinfect and should be cleaned and disinfected regularly and as appropriate.
- Ice should be made from clean potable water. All surfaces that come into contact with ice should be easy to clean and to disinfect and should be cleaned and disinfected regularly and as appropriate, including storage (rooms, bins) and transport (baskets, tubs, boxes) facilities.
- Fish should be purged, where necessary, to reduce gut contents and pollution of fish during further processing.
- Live fish should not be subjected to extremes of heat or cold or sudden variations in temperatures and salinity.

6.10.2.8 *Holding and transportation*

Appropriate holding and transportation techniques should be applied to minimize physical damage and stress to live fish.

- Holding and transportation should be rapid so that fish are not exposed unduly to undesirable high temperatures.
- During holding and transportation, fish should be packed in ice or immersed in ice slush, aiming at keeping the temperature as closer as possible to 0 °C.
- All equipment for fish holding and transportation should be easy to clean and to disinfect and should be cleaned and disinfected regularly and as appropriate.
- Fish should not be transported with other products that might contaminate them.
- Live fish should be handled in such way as to avoid unnecessary stress. Equipment for the transport of live fish should be designed for rapid and efficient handling without causing physical damage or stress.
- Records for transport of fish should be maintained to ensure full product tracing.

6.10.2.9 Storage and transport of live fish

This section is designed for the storage and transportation of live fish originating from aquaculture.

- Only healthy and undamaged fish should be chosen for live storage and transport. Damaged, sick and dead fish should be removed before introduction to the holding or conditioning tanks.
- In order to reduce fish stress, water utilized to fill holding tanks, or to pump fish between holding tanks, or for conditioning fish, should be similar in properties and composition to the water from where the fish is originally taken.
- Water should not be contaminated with either human sewage or industrial pollution. Holding tanks and transportation systems should be designed and operated in a hygienic way to prevent contamination of water and equipment.
- Water in holding and conditioning tanks should be well aerated before fish are transferred into them.
- Where seawater is used in holding or conditioning tanks, for species prone to toxic algae contamination, seawater containing a high level of cell concentrations should be avoided or filtered properly.
- No fish feeding should occur during the storage and transport of live fish. Feeding will pollute the water of holding tanks very quickly, and, in general, fish should not be fed for 24 h before transporting;
- All equipment and facilities should be cleaned and disinfected regularly and as needed.

6.10.2.10 Documentation and records

Where necessary, appropriate records should be kept regarding production site, pond or cage identification, veterinary drugs or medicated feeds given, withdrawal times, harvesting time, and place of destination.

6.10.2.11 Training

Training in fish hygiene and handling is of fundamental importance. All personnel at the fish farm level should be aware of their role and responsibility in protecting fish from contamination or deterioration. Fish handlers should have the necessary knowledge and skills to handle fish hygienically and with proper care. Those who handle strong cleaning chemicals or other potentially hazardous chemicals should be instructed in safe handling techniques.

- Fish farm personnel should be properly trained in hygienic practices to prevent fish contamination and spoilage during production, harvesting and transportation.

- Periodic assessment of the effectiveness of training and instruction programmes should be made, as well as routine supervision and checks to ensure that procedures are being carried out effectively.
- Training instructors should have the necessary knowledge and skills to be able to organize and implement training and extension programmes at the fish farm level.
- Special training programmes should be developed and implemented in endemic areas affected by food-borne trematodiasis (opisthorchiasis, clonorchiasis) aimed at making all persons aware of their essential role in controlling these zoonoses.

6.10.3 Application of HACCP in aquaculture

Application of GAPs is effective for preventing and controlling most food safety and quality hazards at the farm level. That is why many regulatory authorities emphasize mandatory implementation of GAPs as described above, as sufficient for operating fish farms to supply safe and quality fish. However, many experts and the CAC stress that integration of GAPs into HACCP-based systems at the farm level leads to improved cost-effectiveness and real-time prevention and control of hazards. While most control measures and critical limits are well specified in regulatory GAPs, the additional requirements such as hazard analysis, identification of corrective actions, monitoring and HACCP verification allow the farm to take ownership of its food safety and quality programme, respond in real time to safety challenges and develop record-keeping and traceability trails necessary for government or private audit and certification.

The general principles of HACCP and other elements developed in Section 6.3 are also applicable to aquaculture operations and should be consulted for the development of an HACCP plan for aquaculture.

6.10.4 Example of an HACCP plan development for the aquaculture industry

The following is an example that describes how the HACCP principles can be adapted to company VWX, which farms shrimp. This example is provided for illustrative purposes only and should not be applied for a given shrimp farm prior to it being adapted to the prevailing conditions and validated.

6.10.4.1 Introduction

Company VWX is specialized in the production of farmed shrimp destined for export.

The farm has a total area of 300 ha, with pond sizes varying between 3.5 and 5.0 ha, but mainly 4.0 ha. The most recent annual production was 2 600 tonnes of white shrimp (*Litopenaeus vannamei*). The company operates its own hatchery for post-larvae and nauplius production and a shrimp feeds factory. The farmed shrimp is supplied to a nearby processing plant. The source of the water used on the farm is bacteriologically satisfactory. However, being located in an important agricultural region, chemical contamination by agrochemicals is well documented in the region, particularly pesticides (aldrin, dieldrin, DDT and others). Some veterinary drugs (oxytetracycline) are used to control endemic shrimp diseases. Immediately after harvesting, the shrimp are immersed in vats with a mixture of ice and sodium metabisulphite to chill the shrimps and prevent black spot. Iced shrimps are placed in boxes and transported to the shrimp processing plant (15 km from the farm), where a variety of frozen products are produced mainly for the United States market.

Company VWX has developed its HACCP manual in accordance with:

- The Codex Code of Practice for Fish and Fishery Products. CAC/RCP 52-2003, Rev 2008).

- The requirements of the Food and Drug Administration FDA: Title 21 of the Code of Federal Regulations, parts 123 and 1240, entitled “Procedures for the safe and sanitary processing and importing of fish and fishery products”, Federal Register, Volume 60 (No. 242, pp. 65095–65202).

Furthermore, VWX company management has adopted a policy to use HACCP and is also implementing the company’s GAPs and SSOPs, and is controlling quality problems.

6.10.4.2 HACCP team

The HACCP team of company VWX consists of:

- quality control (QC) manager (AB);
- production manager (DE);
- general manager (GH);
- technical adviser (JK);
- pond supervisors (MN, PQ and ST).

This team has expertise in shrimp aquaculture, food safety and quality, and management. The team has developed formal communication channels with food control authorities, extension services, public health authorities and clients to ensure appropriate development of the HACCP manual. Table 83 provides the necessary information on the HACCP team, its qualifications and duties.

TABLE 83

HACCP team of aquaculture company VWX

Name	Background and experience	Title/responsibility	Duties
AB	Food safety degree Certificate of HACCP course from the University of... 10 years experience in food and fish safety and quality	QC manager responsible for the implementation and revision/maintenance of the company's HACCP manual	Participates in the elaboration of the HACCP manual Supervision and implementation of QC activities (sampling, analyses, supervision of corrective actions) Calibration and validation of control methods Supervision of training of company personnel Handling complaints of clients and food control agencies and follow-up on corrective action to these complaints Supervises pest control operations
DE	Aquaculture degree GAP certificate from the College of Fisheries and Aquaculture ... HACCP certificate from the veterinary School ... 7 years experience in food and fish safety and quality	Production manager responsible for the daily running and planning of the production through storage and shipment	Participates in the elaboration of the HACCP manual Plans and supervises production Implements control measures and corrective actions under the guidance of the QC manager Revises the list of suppliers of inputs (feeds, drugs) and packaging
GH	No formal advanced qualification, but practical experience at all levels of the business	General manager in charge of managing the logistics and administration of the company	Draws up the quality and safety policy of the company Approves the HACCP plan and its revisions Commits the company to implementing HACCP Chairs monthly (regular) meetings of the HACCP team to review progress and address issues. Minutes are recorded, filed and distributed to HACCP team
JK	Degree in food safety and quality expert from... Certificate training in shrimp farming from ... 15 years experience in fish and seafood safety and quality	Technical adviser	Supervises the elaboration of HACCP manual and its revision Carries out the yearly audit of the HACCP system Provides relevant information on emerging issues, regulations, safety and quality management guidance
MN, PQ, ST	In-house training	Pond supervisors	Supervise the daily activities during production Implement the control measures under their respective responsibilities

Each team member is responsible for carrying out the duties identified for him/her in the plan, under the supervision of the QC manager who validates all actions necessary for the implementation of the HACCP plan. If needed, the QC manager will refer to the general manager for the implementation of cumbersome and costly actions, presenting the different options and solutions without any compromise on safety and quality, as per company policy. If necessary, the technical adviser is consulted to provide scientific and technical advice as seen fit.

6.10.4.3 Product description

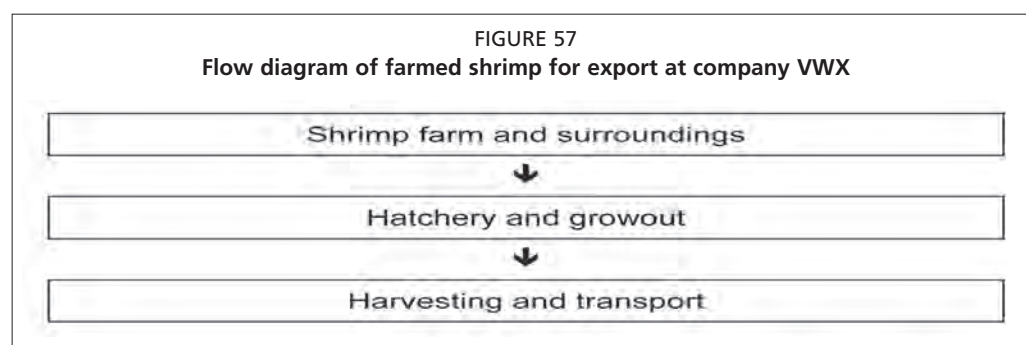
Company VWX produces shrimp (*Litopenaeus vannamei*), which is manually harvested from ponds, immersed in a mixture of potable water, ice and sodium metabisulphite (concentration of 1.25 percent for 1–3 min), placed in plastic boxes mixed with ice and sent by road to a nearby shrimp processing plant.

6.10.4.4 Intended use of the product

The shrimp produced by company VWX is cleaned, graded by size and quality, packed in different retail sizes, and frozen to be sold to international markets, mainly the United States of America. Before consumption, the shrimp are thawed and cooked, roasted or fried. The shrimp are consumed by a large public, with no specific age restriction. The products are labelled to indicate the presence of SO₂ to preserve the shrimp.

6.10.4.5 Construction of the flow diagram

On DD/MM/YYYY, the HACCP team reviewed the current operations used for shrimp production at company VWX, collected the necessary information and constructed the following flow diagram for shrimp production at the company (Figure 57).



6.10.4.6 On-site Confirmation of flow diagram

The HACCP team verified carefully on DD/MM/YYYY the different steps of the flow diagram, and synthesized them into one diagram that was complemented with data and information relevant to HACCP, such as concentration of metabisulphite dip, duration of treatment, water quality, and flow rate. All the collected data were consolidated and used to update the flow diagram.

6.10.4.7 Hazard analysis

Potential hazards that can compromise safety and quality have been studied and analysed by the HACCP team.

To do so, the HACCP team relied on the expertise of its members, the feedback of its clients and that of the food control services, the technical specifications of its

clients and other information available with public health authorities, extension and aquaculture services and on the following authoritative publications:

- Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003, Revision 2008).
- Huss, H.H., Ababouch, L. and Gram, L. 2004. *Assessment and management of seafood safety and quality*. FAO Fisheries Technical Paper No. 444. Rome, FAO. 241 pp.
- Ababouch, L., Gandini, G. and Ryder, J. 2005. Detentions and rejections in international fish trade. FAO Fisheries Technical Paper No. 473. Rome, FAO. 110 pp.

The potential causes of hazards were identified to be either contamination (from the site surroundings, personnel, feed water, ice, birds, and domestic animals), survival to sanitation or non-respect of good practices (use of unauthorized drugs, non-respect of withdrawal time, non-respect of the concentration of sodium metabisulphite and treatment duration). For each cause, the most appropriate control measure was identified.

The results of the hazard analysis, including control measures, are summarized in Table 84. Most of the control measures relate to GAPs or GHPs (also see Appendix 3, Annex A3.I, of this publication). Although different hazards present varying levels of severity and likelihood of occurrence, the HACCP team considered all identified hazards and quality defects equally important and identified control measures to eliminate each hazard or reduce it to acceptable levels, to meet regulatory requirements of importing markets and to avoid rejections or detentions of shipments at international borders or by buyers. The details of hazard analysis are presented in Appendix 3 of this publication.

TABLE 84

Hazard analysis for shrimp production at company VWX

Hazard	Significant	Risk	Control measures
Presence of residues of banned veterinary drugs	yes	+++	Unapproved veterinary drugs in hatchery and ponds shall not be used
Presence of residues of authorized veterinary drugs at levels above MRL	yes	+++	Treatments are supervised by licensed professionals Strict respect of withdrawal times
Shrimp contaminated with pathogenic bacteria	yes	++	Application of GAPs and GHPs
Shrimp contaminated with pesticides	yes	+	Water and soil testing No shrimp farming when risk is high.
High residues of sodium metabisulphites	yes	+	Training of workers in good harvest and post-harvest practices Proper application of these practices

Notes: + = low; ++ = moderate; +++ = high.

6.10.4.8 Identification of critical control points

Once the hazard analysis was carried out, the point where each hazard may appear in the flow diagram and its causes were identified by the HACCP team. Then, each of the flow diagram steps was assessed to determine whether it was a CCP or not. To do this, the HACCP team relied on its expertise and used the decision tree recommended by Codex (Figure 42). The details of CCP identification are presented in Appendix 3 of this publication.

6.10.4.9 Establishment of critical limits

For each identified CCP, the HACCP team designed a critical limit to assess whether the control measure was applied correctly to eliminate the hazard or reduce it to an acceptable level. Again, the HACCP team relied on its expertise, regulatory limits,

guidance and specifications from clients. As much as possible, critical limits were set as operational limits, i.e. limits that indicate a slide towards a loss of control but before the manifestation of the hazard. The details of the critical limits are presented in Appendix 3 of this publication.

6.10.4.10 Development of a monitoring system

Based on its expertise, experience and advice compiled from relevant documents, regulations and clients' specifications, the HACCP team developed a monitoring system to check conformity to the targeted critical limits. The monitoring procedures, including sampling plans where relevant, are described in Appendix 3 (Annex A3.II) of this publication.

6.10.4.11 Identification of corrective actions

Again, relying on its experience and expertise and relevant documents, regulations and clients' specifications, the HACCP team identified corrective actions to be activated when monitoring indicated loss of control, as well as the respective communication and command chains to implement the corrective actions. These actions and the procedure to implement them are described in Appendix 3 of this publication.

6.10.4.12 Verification procedure

Monthly, and after every production cycle, the HACCP team assesses internally all the results of the controls, monitoring and corrective actions, and draws conclusions for the following production cycle.

For the longer time frame, company VWX has set up an annual verification procedure that comprises:

- The evaluation of the monitoring and corrective actions data to assess performance and analyse the reason for any loss of control or for complaints from clients and/or control authorities.
- The results of this analysis are used to update the HACCP manual, identify any internal need for further training and improved practices, performance and maintenance, modify frequency (increase or decrease) of specific monitoring, and the revise list of approved suppliers to eliminate unreliable ones.
- An audit by the technical adviser to assess the performance of each control, monitoring or corrective procedure. The adviser audits the different records, including records for monitoring, calibration and maintenance, training, complaints and reports from clients and control authorities. The adviser prepares a report that is submitted to management and discussed during a meeting with management and the HACCP team. The audit exercise is also used as an opportunity to introduce new procedures, monitoring techniques or critical limits to take into consideration new developments, including new regulatory requirements.

6.10.4.13 Record-keeping procedures

Forms are used to record the results of each monitoring activity and any corrective actions that are implemented. These forms identify who is responsible for the implementation of preventive (control) measures, monitoring and corrective actions and who should validate these actions or be informed of their respective outcome as per the duties described in Table 83. Example forms can be found in Appendix 3 of this publication.

7. International regulatory systems

The increasing demand for fish and fishery products, coupled with technological developments in production, processing, transportation and distribution and the increasing awareness and demand of consumers for safe and high-quality food, has increasingly put food safety and quality assurance issues in the headlines. This has been exacerbated by the recurrent food safety scares since the 1990s.

Amidst the expansion of globalization, internationally traded fish have been subject to close scrutiny for safety and quality. For example, the alert system for food and feed in the European Union (Member Organization) indicated that fish and fishery products were often responsible for a large proportion (up to 25 percent) of food safety and quality alerts in the period 2000–05. Similar safety problems have been reported by the control authorities of other major fish-importing countries. However, several exporting countries challenge these assertions and claim they constitute technical barriers to trade.

International harmonization of safety and quality requirements and equivalence of certification systems can facilitate international fish trade, increase transparency and prevent the use of these requirements as disguised barriers to trade. However, the safety requirements should be based on sound science to provide the appropriate level of consumer protection. Reconciling these objectives requires an international regulatory and technical framework to support the development of harmonized standards and equivalence recognition systems.

What follows is a description of the international regulatory framework for fish safety and quality. It includes how this framework and other scientific developments have been enacted into regulations and operating procedures by the major fish and seafood markets in the world, namely the United States of America, Japan, the European Union (Member Organization), and Australia and New Zealand.

7.1 THE INTERNATIONAL REGULATORY FRAMEWORK

7.1.1 The World Trade Organization (Lahsen Ababouch)

The WTO was established in 1995 as the successor to the GATT (founded after the Second World War). The WTO was established as the final act of the Uruguay Round of negotiations, which began in Punta del Este, Uruguay, in September 1986 and concluded in Marrakech, Morocco, in April 1994. The Uruguay Round was the first to deal with the liberalization of trade in agricultural products, an area excluded from previous rounds of negotiations.

Significant implications for food safety and quality arise from the Final Act of the Uruguay Round, especially from two binding agreements: the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) and the Agreement on Technical Barriers to Trade (the TBT Agreement)⁴².

The SPS Agreement confirms the right of WTO member countries to apply measures necessary to protect human, animal and plant life and health. This right was included in the original 1947 GATT as a general exclusion from the provisions of the agreement provided that “such measures are not applied in a manner which would

⁴² www.wto.org/english/res_e/booksp_e/agrmntseries4_sps_08_e.pdf www.wto.org/english/docs_e/legal_e/17-tbt.pdf

constitute a means of arbitrary or unjustifiable discrimination between countries where the same conditions prevail, or a disguised restriction on international trade". Despite this general condition for the application of national measures to protect human, animal and plant life and health, such measures had become effective trade barriers, whether by design or accident.

The purpose of the SPS Agreement is to ensure that measures established by governments to protect human, animal and plant life and health in the agriculture sector, including fisheries, are consistent with obligations prohibiting arbitrary or unjustifiable discrimination on trade between countries where the same conditions prevail and are not disguised restrictions on international trade. It requires that, with regard to food safety measures, WTO members base their national measures on international standards, guidelines and other recommendations adopted by the CAC where they exist. (This does not prevent a member country from adopting stricter measures if there is a scientific justification for doing so, or if the level of protection afforded by the Codex standards is inconsistent with the level of protection generally applied and deemed appropriate by the country concerned.)

The SPS Agreement states that any measures taken that conform to international Codex standards, guidelines or recommendations are deemed to be appropriate, necessary and not discriminatory. In addition, the SPS Agreement calls for a programme of harmonization based on international standards. This work is guided by the WTO Committee on SPS Measures. Membership includes representatives of the CAC, the International Office of Epizootics (OIE), which deals with animal health (including fish), and the International Plant Protection Convention, which deals with plant protection.

Finally, the SPS Agreement requires that SPS measures shall be based on an assessment of the risks to humans, animal and plant life and health using internationally accepted risk assessment techniques. Risk assessment should take into account the available scientific evidence, the relevant processes and production methods, the inspection/sampling/testing methods, the prevalence of specific illnesses and other matters of relevance.

The TBT Agreement is a revision of the agreement of the same name, first developed under the Tokyo Round of negotiations (1973–79). The objective of the TBT Agreement is to prevent the use of national or regional technical regulations and standards as unjustified technical barriers to trade. The agreement covers standards relating to all types of products including industrial products and quality requirements for foods (except requirements related to SPS measures). It includes numerous measures designed to protect the consumer against deception and economic fraud.

The TBT Agreement basically provides that standards and technical regulations must have a legitimate purpose and that the impact or the cost of implementing the standard must be proportional to the purpose of the standard. It also states that, if there are two or more ways of achieving the same objective, the least trade-restrictive alternative should be followed. The agreement also places emphasis on international standards – WTO members are encouraged to use international standards or parts of them except where the international standard would be ineffective or inappropriate in the national situation.

The SPS and TBT Agreements call on WTO member countries to:

- promote international harmonization and equivalence agreements;
- promote the use of scientifically sound risk assessment to develop SPS measures;
- facilitate the provision of technical assistance, especially to developing countries, either bilaterally or through the appropriate international organizations;

- take into consideration the needs of developing countries, especially the least-developed countries, when preparing and implementing SPS and quality measures.

The aspects of food standards that TBT requirements specifically cover are quality provisions, nutritional requirements, labelling, packaging and product content regulations, and methods of analysis. Unlike the SPS Agreement, the TBT Agreement does not specifically name international standard-setting bodies whose standards are to be used as benchmarks for judging compliance with the provisions of the TBT Agreement.

7.1.2 The Codex Alimentarius Commission (Iddya Karunasagar)

Since 1962, the Codex Alimentarius Commission (CAC)⁴³ has been responsible for implementing the Joint FAO/WHO Food Standards Programme. The CAC's primary objectives are the protection of the health of consumers, the assurance of fair practices in food trade, and the coordination of the work on food standards.

The CAC is an intergovernmental body with a membership of 184 member countries and one member organization (as of September 2012). In addition, observers from international intergovernmental organizations (e.g. OIE, WTO and the International Atomic Energy Agency) and international NGOs (i.e. scientific organizations, food industry, food trade and consumer associations) may attend sessions of the CAC and of its subsidiary bodies. An executive committee, six regional coordinating committees and a secretariat assist the CAC in administering its work programme and other related activities (Figure 58).

The work of the CAC is divided between three basic types of committees:

- general subject committees that deal with general principles, hygiene, veterinary drugs, pesticides, food additives, contaminants, labelling, methods of analysis and sampling, nutrition and foods for special dietary uses and import/export inspection and certification systems;
- commodity committees that deal with a specific type of food class or group, such as dairy and dairy products, fats and oils, or fish and fishery products;
- ad hoc intergovernmental task forces (whose number is variable) that are established to deal with specific issues within a limited time frame (usually five years).

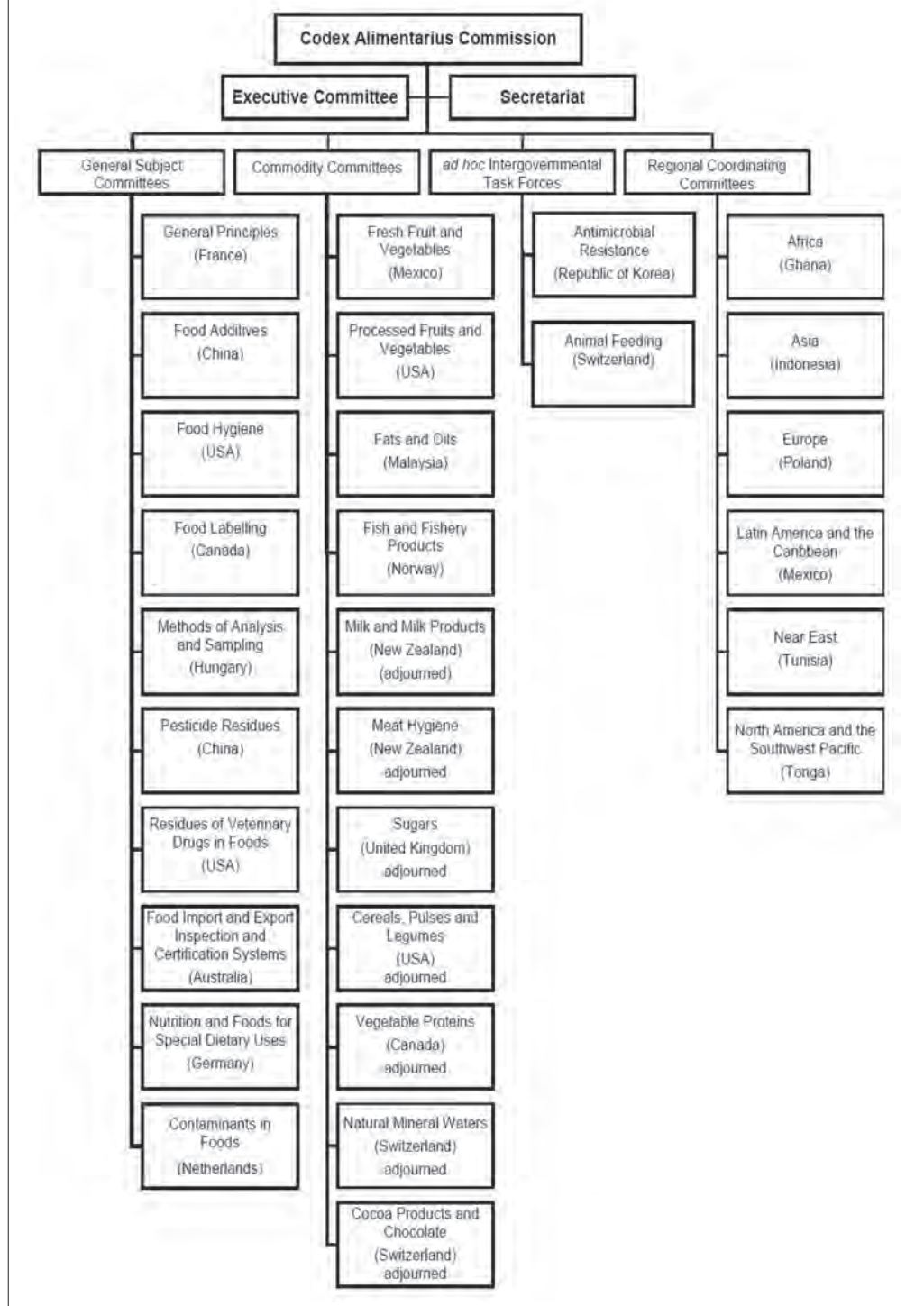
The work of the committees on food hygiene, contaminants, fish and fishery products, veterinary drugs and import/export inspection and certification systems are of paramount interest to the safety and quality of internationally traded fish and fishery products.

In the environment of the WTO agreements, concluded at the end of the Uruguay Round of multilateral trade negotiations, the work of the CAC has taken on unprecedented importance with respect to consumer protection and international food trade. Codex standards, guidelines and codes of practice relating to food safety are specifically recognized by the WTO SPS Agreement, including the MRLs for pesticides and veterinary drugs, the maximum limits of food additives, the maximum levels of contaminants, and food hygiene requirements of Codex standards.

In the specific area of food hygiene, the CAC has revised its main document on food hygiene (CAC/RCP 1-1969, Revision 2003) to incorporate the principles of risk analysis and to include specific references to the HACCP system.

⁴³ www.codexalimentarius.org

FIGURE 58
Joint FAO/WHO Food Standards Programme



Since 1992, the Codex Committee on Fish and Fishery Products (CCFFP) has been developing a new Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003) (CPFFP) that is based on risk analysis principles and that merges and updates the previous individual codes of practice. All sections of the CPFFP aim at providing a user-friendly document with background information and guidance.

It will be completed within a few years and contain the following general and specific sections:

- Section 1. Scope
- Section 2. Definitions
- Section 3. Prerequisite Programme
- Section 4. General Considerations for the Handling of Fresh Fish and Shellfish and other Aquatic Invertebrates
- Section 5. Hazard Analysis Critical Control Point (HACCP) and Defect Action Point (DAP) Analysis
- Section 6. Aquaculture Production
- Section 7. Live and Raw Bivalve Molluscs
- Section 8. Processing of Fresh, Frozen and Minced Fish
- Section 9. Processing of Frozen Surimi
- Section 10. Processing of Quick Frozen Coated Fish Products
- Section 11. Processing of Salted and Dried Salted Fish
- Section 12. Smoked Fish (under development)
- Section 13. (a) Lobsters (b) Crabs (both under development)
- Section 14. Processing of Shrimps and Prawns
- Section 15. Processing of Cephalopods
- Section 16. Processing of Canned Fish and Shellfish
- Section 17. Transportation
- Section 18. Retail

The CPFFP is designed to assist all those engaged in the handling and production of fish, shellfish and their products, or concerned with their control, storage, distribution, export, import and sale to:

- attain safe and wholesome products, which can be sold on national or international markets;
- meet the requirements of the Codex standards, both in terms of health and safety requirements and essential quality, composition and labelling provisions.

The Codex Alimentarius includes a number of product standards, such as those for dried fish, salted fish, quick-frozen fish, and canned fish. Codex has also adopted a Model Certificate for Fish and Fishery Products (CAC/GL 48-2004)⁴⁴.

In addition, the Recommended International Code of Practice for the Processing and Handling of Quick Frozen Foods, totally revised in 2008, provides useful advice for the safe handling for quick-frozen fishery products (CAC/RCP 8-1976)⁴⁵.

The Codex standards and related texts are voluntary by nature – they can become mandatory only when converted into national legislation or regulations. However, under the SPS and TBT Agreements, the Codex standards play a role as the international benchmark for harmonization and may be used for reference when settling trade disputes (Box 3). There is concern that this may bring more politics into the CAC. The CAC's unchanging role should be to act as an international risk manager and to continue to provide sound and science-based recommendations to its member countries. In doing so, the CAC depends on the scientific advice in risk assessment provided by expert bodies convened by FAO and WHO.

⁴⁴ www.codexalimentarius.net/download/standards/10127/CXG_048e.pdf

⁴⁵ www.codexalimentarius.net/download/standards/285/CXP_008e.pdf

BOX 3

Example of a WTO trade dispute: the sardine case

Codex standards have already been used as the benchmark in international trade disputes, and they are expected to play an increasing role. Although such disputes generally involve a limited number of countries, they have a direct impact on international fish trade.

A dispute regarding canned sardine labelling illustrates the importance of both WTO agreements and the Codex Alimentarius.

The dispute arose when Peru started to export canned products from the clupeid species *Sardinops sagax* to the then European Communities; the canned products were labelled as “Pacific sardines”. According to the European Commission (EC) regulation in force at that time, the term “sardine” was exclusively reserved for the species *Sardina pilchardus*. The *Sardinops* products were not allowed to enter the market of the European Communities as “Pacific sardine”.

Peru and the European Communities held consultations but failed to reach a mutually satisfactory solution. Then, Peru requested the WTO Dispute Settlement Body to establish a panel to examine the issue.

Peru claimed that, according to the relevant Codex standard for canned sardine and sardine-type products, the species “*Sardinops sagax sagax*” is listed among those species that can be traded as “sardines”. Peru, therefore, considered that the EC Regulation constituted an unjustifiable barrier to trade, and, hence, in breach of Articles 2 and 12 of the TBT Agreement.

The Panel Report concluded that the EC Regulation was inconsistent with Article 2.4 of the TBT Agreement. The WTO Appellate Body upheld the Panel’s finding that Codex Stan 94 (1981) is a “relevant international standard” and is effective and efficient in pursuing the legitimate objectives of promoting market transparency, consumer protection and fair competition.

Finally, the EC and Peru reached a mutually agreed solution to the dispute, which was notified to the Dispute Settlement Body. The EC consequently amended its regulation in order to bring it into conformity with Codex and the TBT Agreement. The dispute settlement took two years to be resolved. The revised EC regulation includes in particular:

- a definition of “preserved sardine-type products”, which may be prepared from various clupeiforme species (same list as in the Codex standard);
- preserved sardine-type products may be marketed in the European Communities under a trade description consisting of the word “sardines” joined together with the scientific name of the species.

7.1.3 The FAO Code of Conduct for Responsible Fisheries (Lahsen Ababouch)

In recent decades, world fisheries have become a market-driven, dynamically developing sector of the food industry, and coastal States have striven to take advantage of their new opportunities by investing in modern fishing fleets and processing factories in response to the growing international demand for fish and fishery products. However, by the late 1980s, it had already become clear that fisheries resources could no longer sustain such rapid and often uncontrolled exploitation, and that new approaches to fisheries management embracing conservation and environmental considerations were urgently needed.

At its Nineteenth Session (in March 1991), COFI called for the development of new concepts that would lead to responsible, sustained fisheries. Subsequently, the International Conference on Responsible Fishing, held in 1992 in Cancún (Mexico), further requested FAO to prepare an international code of conduct to address these concerns. The outcome of this Conference, particularly the Declaration of Cancún, was

an important contribution to the 1992 United Nations Conference on Environment and Development (UNCED), in particular its Agenda 21.

Noting these and other important developments in world fisheries, the FAO Governing Bodies recommended the formulation of a global Code of Conduct for Responsible Fisheries (the Code), which would be consistent with these instruments and, in a non-mandatory manner, establish principles and standards applicable to the conservation, management and development of all fisheries. The Code, which was unanimously adopted on 31 October 1995 by the Twenty-eighth Session of the FAO Conference, provides a necessary framework for national and international efforts to ensure sustainable exploitation of aquatic living resources in harmony with the environment (FAO, 1995).

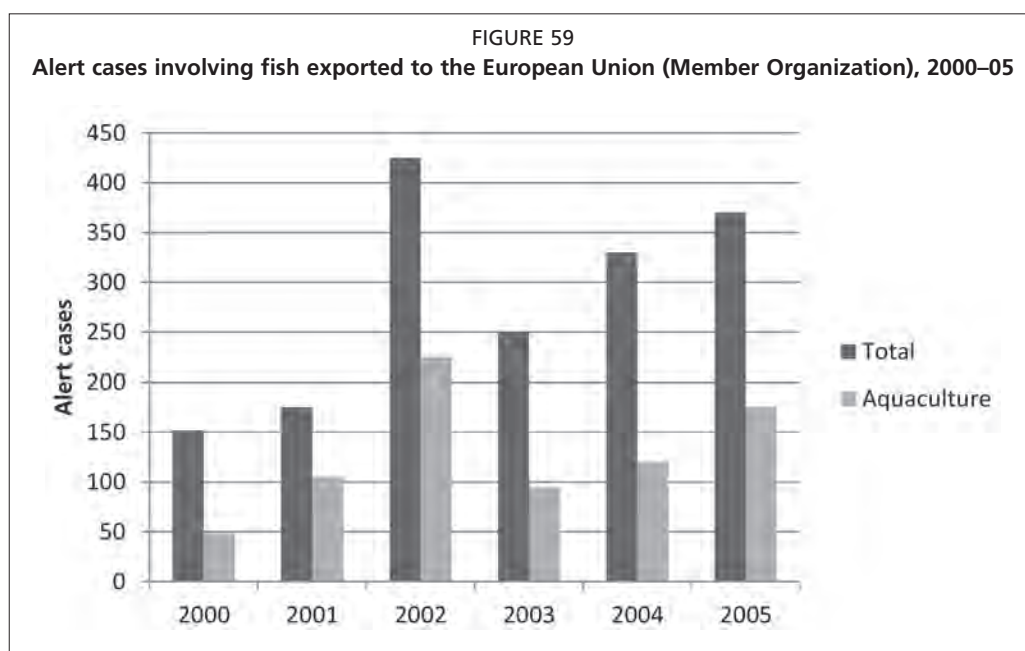
Article 6 (General principles, provisions 6.7 and 6.140) and article 11 (Post-harvest practices and trade) are of particular relevance to fish trade, safety and quality. Provisions 11.1.2, 11.1.3 and 11.1.4 encourage States to establish and maintain effective national safety and quality assurance systems, to promote the implementation of the CAC standards and codes of practice and to cooperate to achieve harmonization or mutual recognition, or both, of national sanitary measures and certification programmes.

The same Twenty-eighth Session of the FAO Conference requested the elaboration of technical guidelines in support of the implementation of the Code in collaboration with Members and relevant organizations. FAO has published technical guidelines for responsible fish utilization (FAO, 1998) and for responsible fish trade (FAO, 2009b). For safety and quality, both sets of guidelines confirm the basic principles of the SPS and TBT Agreements and highlight the necessity to base safety and quality requirements on the standards, guidelines and codes of practice of the CAC.

Because of the increasing contribution of aquaculture to the supply of fish for human consumption, safety and quality issues in this sector have been receiving special attention. For example, aquaculture products were involved in 28–63 percent of alert cases in the European Union (Member Organization) in the period 2000–05, mainly because of the presence of high residues of veterinary drugs, unauthorized chemicals and bacterial pathogens. For the 2005 alone, 177 alert cases were due to aquaculture products that contained bacterial pathogens (37 percent), nitrofurans (27 percent), green malachite (20 percent), excess residues of sulphites (13 percent) and unacceptable residues of veterinary drugs (3 percent) (Figure 59) (Ababouch, 2012).

These concerns have spurred the development of private standards and certification schemes to be used in business-to-business transactions between suppliers and buyers. However, this has led to the proliferation of market standards and confusion of consumers and producers as to which certification programmes carry the most value. It also raises questions about which certification programmes best serve consumer protection, the environment, the public and the producers.

Consequently, in 2006, FAO was requested to develop international guidelines for certification in aquaculture. These guidelines were finalized and adopted in 2011 by the Twenty-ninth Session of COFI. They can be used by government, private-sector or other organizations to develop transparent and reliable certification standards and schemes in aquaculture.



7.1.4 World Organisation for Animal Health (Rohana Subasinghe and Melba Reantaso)

The World Organisation for Animal Health,⁴⁶ an intergovernmental organization created on 25 January 1924 as the Office International des Epizooties (OIE) and based in Paris, had 178 member countries and territories as of September 2012. The OIE has the objectives of ensuring transparency in the global animal disease and zoonosis situation by each member country undertaking to report the animal diseases that it detects on its territory. The information, which also includes diseases transmissible to humans, is disseminated by the OIE to other countries, immediately or periodically depending on the seriousness of the diseases, so that countries can take the necessary preventive actions. The latest scientific information on animal disease control is also collected by the OIE and such information is then made available to member countries and territories to help improve the methods used to control and eradicate these diseases. Technical support is provided by the OIE to member countries requesting assistance with animal disease control and eradication operations, including diseases transmissible to humans. With regard to aquatic animal diseases, the main normative works produced by the OIE are the Aquatic Animal Health Code⁴⁷ and the Manual of Diagnostic Tests for Aquatic Animals.⁴⁸ Standards issued by the OIE are recognized by the WTO as reference international sanitary rules.

The OIE Aquatic Animal Health Code (the Aquatic Code) sets out standards for the improvement of aquatic animal health and welfare and veterinary public health worldwide, including through standards for safe international trade in aquatic animals (amphibians, crustaceans, fish and molluscs) and their products. The health measures in the Aquatic Code should be used by the veterinary authorities of importing and exporting countries to provide for early detection and reporting and to control agents pathogenic to aquatic animals and, in the case of zoonotic diseases, to humans, and to prevent their transfer via international trade in aquatic animals and aquatic animal products, while avoiding unjustified sanitary barriers to trade.

⁴⁶ www.oie.int

⁴⁷ www.oie.int/international-standard-setting/aquatic-code/

⁴⁸ www.oie.int/manual-of-diagnostic-tests-for-aquatic-animals/

The Aquatic Code deals with general obligations related to certification and certification procedures during trade (import and export) and movement of aquatic animals and animal products. An international aquatic animal health certificate is a document, drawn up by the exporting country in accordance with the Aquatic Code, describing the aquatic animal health requirements for the exported commodity. The assurance given to the importing country that diseases will not be introduced through the importation of aquatic animals or aquatic animal products depends on the quality of the exporting country's aquatic animal health infrastructure and the rigour with which international aquatic animal health certificates are issued in the exporting country. These international aquatic animal health certificates are intended to facilitate safe trade and should not be used to impede it by imposing unjustified health conditions. In all cases, the exporting country and the importing country should refer to the health conditions recommended in the Aquatic Code before agreeing on the terms of the certificate. They should also respect their rights and obligations under the SPS Agreement.

One of the key objectives of the Aquatic Code is to help OIE members trade safely in aquatic animals and animal products by developing relevant aquatic animal health measures. These recommendations address aquatic animal health hazards and food safety hazards in aquatic animal feed. A key objective is to prevent the spread, via aquatic animal feed, of diseases from an infected area to a disease-free area. These recommendations complement the CAC Code of Practice on Good Animal Feeding (CAC/RCP 54-2004). The FAO Technical Guidelines for Responsible Fisheries *Aquaculture Development: 1. Good Aquaculture Feed Manufacturing Practice* (FAO, 2001) and the FAO / International Feed Industry Federation *Good Practices for the Feed Industry* (FAO/IFIF, 2010) may be relevant sources of guidance.

The Aquatic Code also provides guidance for members to address appropriately the selection and spread of resistant micro-organisms and antimicrobial resistance genes due to the use of antimicrobial agents in aquatic animals. The Aquatic Code also provides principles on the responsible and prudent use of antimicrobial agents in aquatic animals, with the aim of protecting both animal and human health.

Another aspect that the Aquatic Code addresses is fish welfare. The OIE is currently developing recommendations for the welfare of farmed fish (excluding ornamental species) during transport, slaughter, and destruction for disease control purposes.

In response to the demand from consumers worldwide for safe food, the OIE is working with relevant organizations to reduce food-borne risks to human health due to hazards arising from animal production. In this context, a hazard is defined as a biological, chemical or physical agent in food with the potential to cause an adverse health effect in humans, whether or not it causes disease in animals. The Third OIE Strategic Plan (2001–2005)⁴⁹ recommended that “OIE should be more active in the area of public health and consumer protection” and noted that this should include “zoonoses and diseases transmissible to humans through food, whether or not animals are affected by such diseases”, with the object of improving the safety of the “food production to consumption continuum” worldwide.

In 2002, the director-general of the OIE established a permanent Working Group on Animal Production Food Safety (APFSWG) to coordinate the food safety activities of the OIE. The APFSWG's membership includes internationally recognized experts from FAO, WHO and the CAC, and reflects a broad geographical basis.

The Fourth OIE Strategic Plan (2006–2010), firmly established the OIE's role and work programme relevant to food safety in animal production and the arrangements for cooperation with the CAC in the provision to member governments and other interested parties of consistent, coherent and complementary advice on the

⁴⁹ www.oie.int/doc/ged/D2128.PDF

management of food safety risks from the farm to the fork. The Fifth OIE Strategic Plan (2011–2015) confirms the mandate of the APFSWG to continue working with relevant organizations, especially the CAC, FAO and WHO, with the goal of reducing risks to human health due to hazards arising from animal products. The APFSWG will continue its programme for the development of standards relevant to the pre-slaughter sector of the food chain, with a primary focus on food safety measures applicable at the farm level. This work covers pathogens and other hazards that do not normally cause disease in animals.

7.2 EUROPEAN UNION (MEMBER ORGANIZATION) (ALAN REILLY)

7.2.1 Introduction

Following the publication of the White Paper on Food Safety in 2000,⁵⁰ and the subsequent review of the food hygiene regulations of the European Union (Member Organization), new rules, which were accompanied by regulations on the organization of official food controls, came into force in 2006. The approach taken in the legislation is to separate aspects of food hygiene from animal health, and it aims to remove any duplication and inconsistencies that could cause difficulties for both businesses and regulatory authorities. The legislation focuses on the need to protect public health in a way that is effective, proportionate and based on risk.

A key aspect of the legislation is that all food and feed business operators, from farmers and processors to retailers and caterers, have principal responsibility for ensuring that food placed on the market in the European Union (Member Organization) meets the required food safety standards. The regulations apply at every stage in the food chain, including primary production (i.e. farming, fishing and aquaculture) in line with the “farm to fork” approach to food safety in the European Union (Member Organization). The regulations apply to food businesses that catch and farm fish and crustaceans, that farm and handle live bivalve molluscs and those handling and processing fish and fishery products. The responsibilities of food business operators are clearly set out in the regulations, which also require appropriate own-checks to be carried out and include the taking of samples by industry to ensure the marketing of safe fishery products. The regulations also include provisions for guides to good practice to be developed by industry with support from other stakeholders. The legislation applies directly to food businesses, and the effect the legislation will have depends on the size and nature of the business.

The food hygiene regulations constitute a complementary set of rules to harmonize food safety measures in the European Union (Member Organization). They are a suite of several regulations including Regulation EC/852/2004 (EC, 2004c), which lays down the general hygiene requirements for all food business operators, and Regulation EC/853/2004 (EC, 2004a), which lays down additional specific requirements for food businesses dealing with foods of animal origin, including live bivalve molluscs and fishery products. Regulation EC/854/2004 (EC, 2004b) lays down the official controls for foods of animal origin. The basis for the regulations is set down by the General Food Law Regulation EC/178/2002 (EC, 2002a), which provides a framework to ensure a coherent approach in the development of food legislation. The General Food Law Regulation set down definitions, principles and obligations covering all stages of food and feed production and distribution. Other related recent legislation includes the regulation on microbiological criteria for foodstuffs, the regulation on official feed and food controls, and the regulation on feed hygiene.

⁵⁰ http://ec.europa.eu/food/food/intro/white_paper_en.htm

7.2.2 Exporting fish and fishery products to the market of the European Union (Member Organization)

For all food and feed, including fish and fishery products, the general principle is that the product meets or is equivalent to the standards of the European Union (Member Organization). In addition, under current arrangements, in order to export products of animal origin to the European Union (Member Organization), the country must be approved for the relevant commodity and the products must originate in an establishment that is approved to export to the European Union (Member Organization).⁵¹ Lists are maintained at the level of European Union (Member Organization) of countries and establishments from which imports are permitted.⁵² Countries and establishments approved in this manner are commonly referred to as “listed”. In order to be listed, the third country concerned must provide guarantees that exports to the European Union (Member Organization) meet, or are equivalent to, the standards prescribed in the relevant legislation of the European Union (Member Organization).

All consignments of live animals and products of animal origin introduced into the territory of the European Union (Member Organization) must be presented at an approved border inspection post⁵³ of the European Union (Member Organization) to undergo mandatory veterinary checks, and they must be accompanied by a health certificate.

7.2.3 Food business registration and approval

Under the current legislation, primary producers involved in fishing and aquaculture must be registered with the national competent authority as food business operators. Operators will need to register before starting at a new location and will also need to inform the competent authority of the nature of their business. Moreover, establishments must be approved if they handle products of animal origin for which specific hygiene conditions are laid down in the legislation of the European Union (Member Organization). This includes those handling live bivalve molluscs and fishery products. Premises in compliance with the new regulations should be issued an approval number that must accompany all shipment documentation.

7.2.4 Identification marking and labelling

A food business must apply its identification mark before the product leaves the establishment of production. This mark must be legible, indelible and clearly visible for inspection. It must show the name or two-letter code of the country (for example, IE for Ireland) and the approval number of the premises.

7.2.5 Primary production

The farm-to-fork approach of the legislation of the European Union (Member Organization) embraces primary production, and the general principles of food hygiene legislation now extend to all operations engaged in the primary production of food.

“Primary production” is defined as the production, rearing or growing of primary products up to and including harvesting, hunting, fishing, milking and all stages of animal production prior to slaughter. Fish and shellfish farmers are primary producers and are required to follow good farming practices and manage their operations as set out in Annex 1 of Regulation EC/852/2004 (EC, 2004c). Primary producers are not required to implement an HACCP system.

⁵¹ http://ec.europa.eu/food/international/trade/guide_thirdcountries2006_en.pdf

⁵² http://ec.europa.eu/food/food/biosafety/establishments/third_country/index_en.htm

⁵³ http://ec.europa.eu/food/animal/bips/approved_bips_en.htm

In practical terms, the requirements for primary producers amount to the application of good standards of basic hygiene. Primary producers must ensure that hazards are acceptably controlled and that they comply with existing legislation. Under current rules, primary producers need to take steps, for example, to:

- prevent contamination arising from water, soil, feed, veterinary products, waste, etc;
- keep animals (fish) intended to be placed on the market for human consumption clean;
- take account of results from tests relevant to animal and human health;
- use medicines appropriately.

The requirements for food business operators in Annex 1 of Regulation EC/852/2004 (EC, 2004c) also apply to certain associated activities that include:

- the transport, handling and storage of primary products at the place of production, where their nature has not been substantially altered;
- the transport of live animals, where this is necessary;
- transport, from the place of production to an establishment, of products of plant origin, fishery products and wild game, where their nature has not been substantially altered.

7.2.6 General requirements for food business operators

Food business operators, such as fish processors and manufacturers, carrying out activities other than primary production have to comply with the general hygiene provisions of Annex II of Regulation EC/852/2004 (EC, 2004c). This annex sets out the details for the hygiene requirements for:

- food premises, including outside areas and sites;
- transport conditions;
- equipment;
- food waste;
- water supply;
- personal hygiene of persons in contact with food;
- food;
- wrapping and packaging;
- heat treatment, which may be used to process certain foodstuffs;
- training of food workers.

7.2.7 Requirements for live bivalve molluscs and fishery products

Food business operators making or handling products of animal origin must also comply with the provisions of Regulation EC/853/2004 and where appropriate, certain specific rules concerning microbiological criteria for foodstuffs, temperature control and compliance with the cold chain, and sampling and analysis requirements. Foods of animal origin include live bivalve molluscs and fishery products. The provisions of Regulation EC/853/2004 apply to unprocessed and processed products of animal origin, but do not apply to composite foods, i.e. foods containing both products of plant origin and processed products of animal origin (EC, 2004a). However, the processed products of animal origin used in composite foods must be obtained and handled in accordance with the requirements of Regulation (EC) No 853/2004.

Regulation EC/854/2004 lays down specific rules for the organization of official controls on products of animal origin intended for human consumption (EC, 2004b). This regulation supplements Regulation EC/852/2004 on hygiene of foodstuffs and Regulation EC/853/2004 on specific hygiene rules for foodstuffs of animal origin (EC, 2004c, 2004a). This official control regulation gives details of the controls to be carried out on live bivalve molluscs and fishery products.

Details in relation to the approval of establishments and the withdrawal of approval if serious deficiencies are identified on the part of the food business operator are also set out in Regulation EC/854/2004 (EC, 2004b). Food business operators must provide authorized officers with all assistance needed to carry out the controls, notably as regards access to premises and the presentation of documentation or records. The official controls include audits of GHPs and HACCP principles, as well as specific controls that have requirements determined by sector (including live bivalve molluscs and fishery products).

Regulation EC/2074/2005 sets out implementing measures for certain provisions of the hygiene regulations that apply to fish and fishery products (EC, 2008). This regulation includes rules for fishery products encompassing detection of parasites, maximum levels for total volatile nitrogen for certain species as a determinant of “fitness”, testing methods for marine biotoxins and labelling with cooking instructions for specified fish.

7.2.8 Live bivalve molluscs

Harvested live bivalve molluscs intended for human consumption must comply with high health standards applicable at all stages of the production chain. With the exception of the provisions on purification, the rules also apply to live echinoderms, tunicates and marine gastropods. The regulations include provisions for cooperation by food business operators in the classification system. Approved dispatch and purification centres are now required to establish an HACCP system as explained below.

Regulation EC/853/2004 (EC, 2004a) specifies requirements for the following areas:

- production of live bivalve molluscs from Class A, B or C production areas;
- harvesting of molluscs and their transport to a dispatch or purification centre, relaying area or processing plant;
- relaying of molluscs in approved areas under optimal conditions of traceability and purification;
- essential equipment and hygiene conditions in dispatch and purification centres;
- health standards applicable to live bivalve molluscs: freshness and viability; microbiological criteria, evaluation of the presence of marine biotoxins and harmful substances in relation to the permissible daily intake;
- health marking, wrapping, labelling, storage and transport of live bivalve molluscs;
- rules applicable to scallops harvested outside classified areas.

Regulation EC/854/2004 (EC, 2004b) specifies that new production areas require a sanitary survey and the establishment of a representative sampling programme based on the sanitary survey data.

7.2.9 Fishery products

Specific requirements for fish and fishery products cover the following elements:

- equipment and facilities on fishing vessels, factory vessels and freezer vessels: areas for receiving products taken on board, work and storage areas, refrigeration and freezing installations, pumping of waste and disinfection;
- hygiene on board fishing vessels, factory vessels and freezer vessels: cleanliness, protection from any form of contamination, washing with water and cold treatment;
- conditions of hygiene during and after the landing of fishery products: protection against any form of contamination, equipment used, auction and wholesale markets;
- fresh and frozen products, mechanically separated fish flesh, parasites harmful to human health (visual examination), and cooked crustaceans and molluscs;

- processed fishery products;
- health standards applicable to fishery products: evaluation of the presence of substances and toxins harmful to human health;
- wrapping, packaging, storage and transport of fishery products.

Record-keeping – under current regulations, food business operators are required to keep records relevant to food safety, including:

- the nature and origin of animal/fish feed (if used);
- any veterinary products administered and their withdrawal dates (if used);
- any occurrence of disease that may affect food safety;
- the results of any analyses carried out;
- the health status of the animals prior to slaughter.

7.2.10 Hazard Analysis and Critical Control Point (HACCP)

The legislation of the European Union (Member Organization) on hygiene regulations requires food business operators (except primary producers) to put in place, implement and maintain a permanent procedure, or procedures, based on the principles of HACCP. The requirements take a risk-based approach and can be applied flexibly in all food businesses regardless of the size or nature of the business.

7.2.11 Training

Food business operators are responsible for ensuring that food handlers have received adequate instruction and/or training in food hygiene to enable them to handle food safely. Training should be appropriate to the tasks of staff in a particular food business and be appropriate for the work to be carried out. Training can be achieved in different ways. These include in-house training, the organization of training courses, information campaigns from professional organizations or from regulatory authorities, guides to good practice, etc. With regard to HACCP training for staff in small businesses, it must be kept in mind that such training should be proportionate to the size and the nature of the business and should relate to the way that HACCP is applied in the food business. If guides to good practice for hygiene and for the application of HACCP principles are used, training should aim to make staff familiar with the content of such guides.

7.2.12 Microbiological criteria of foodstuffs

The Microbiological Criteria for Foodstuffs Regulation (EC/2073/2005a) includes limits for certain micro-organisms in specified foodstuffs and sets down limits for food safety criteria and process hygiene criteria (EC, 2005). The regulation sets down the *E. coli* and *Salmonella* limits for placing live bivalve molluscs and live echinoderms, tunicates and gastropods on the market for human consumption. It also sets down limits for fishery products for the following:

- *Listeria monocytogenes* for RTE food;
- *Salmonella* for cooked crustaceans and molluscan shellfish;
- Histamine for species associated with high amounts of histidine;
- *E. coli* and coagulase-positive staphylococci for shelled and shucked products of cooked crustaceans and molluscan shellfish (process criteria).

Regulation EC/2073/2005 contains detailed controls encompassing sampling and analysis requirements (EC, 2005a). It is structured so that it can be applied flexibly in all food businesses, regardless of their type or size. Food business operators should apply the criteria within the framework of procedures based on HACCP principles. The criteria can be used by food business operators to validate and verify their food safety management procedures and when assessing the acceptability of foodstuffs, or their manufacturing, handling and distribution processes.

7.2.13 Traceability and withdrawal of food products

In accordance with Regulation EC/178/2002, food business operators must set up traceability systems and procedures for ingredients, foodstuffs and, where appropriate, animals used for food production (EC, 2002a). Similarly, where a food business operator identifies that a foodstuff presents a serious risk to health, it shall immediately withdraw that foodstuff from the market and inform users and the relevant competent authority.

7.2.14 Animal health rules

Council Directive 2002/99/EC lays down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption (EC, 2002b).

Council Directive 2006/88/EC covers health requirements for aquaculture animals and controls of certain fish and bivalve diseases (EC, 2006c). The main aim of the directive is to raise standards of aquaculture health throughout the European Union (Member Organization) and to control the spread of disease while maintaining freedom for trade. While its focus is primarily aquaculture production businesses, it also contains provisions relating to stocked fisheries for angling, installations that keep fish but do not intend to market them, smaller-scale farmers who produce directly for human consumption, and fish kept for ornamental purposes.

7.2.15 Animal and fish feeds

Regulation EC/183/2005 lays down the requirements for feed hygiene (EC, 2005b). It ensures that feed safety is considered at all stages of the feed chain that may have an impact on feed and food safety. The regulation requires the compulsory registration of all feed business establishments and the approval of those operators that are involved in the production of certain feed additives, pre-mixtures and compound feeding stuff. It also requires the application of GHPs at all levels of feed production and the introduction of the HACCP principles for the feed business operators other than at the level of primary production.

The regulation provides for a framework for the European Union (Member Organization) for guides to good practice in feed production, and such a guide has been published.⁵⁴

7.2.16 Residue monitoring programmes

Regulations of the European Union (Member Organization) include requirements for a wide range of food monitoring for residues of veterinary drugs, pesticides and chemical contaminants. Much of the legislation in this area refers to food animal production that would include farmed fish but does not always specifically refer to fish. In European Union (Member Organization), complex regulations exist for the approval of use of medicines for prevention or cure of animal diseases, for setting MRLs of permitted animal remedies and to check for compliance with these MRLs, for monitoring for levels of banned animal remedies, for monitoring levels of pesticides in farmed fish and for monitoring levels of chemical contaminants such as dioxins and heavy metals in fishery products. Methods of analyses and sampling plans for use during monitoring are also included in the regulations.

Among the key regulations, Directive 2001/82/EC (EC, 2001c) stipulates that veterinary medicinal products can only be authorized or used in food producing animals if pharmacologically active substances contained therein have been assessed as safe according to Regulation EC 470/2009 which establishes MRLs for these products (EC, 2009b). Directive 1996/23/EC on residues monitoring contains specific

⁵⁴ http://ec.europa.eu/food/food/animalnutrition/feedhygiene/efmc_1_0_en.pdf

requirements for the control of pharmacologically active substances that may be used as veterinary medicinal products in food animal production (EC, 1996a). This includes primarily sampling and investigation procedures, requirements on the documentation of use, indication for sanctions in case of non-compliance, requirements for targeted investigations and for the establishment and reporting of monitoring programmes. Directive 1996/22/EC prohibits the use of certain substances in food-producing animals (EC, 1996b).

Sample frequencies for testing farmed fish for compliance with regulations of the European Union (Member Organization) have been published by the European Commission.⁵⁵ For those countries where fish and fishery products from any farm are eligible to be exported to the European Union (Member Organization), the proportion of animals sampled should be taken relative to the annual national production figures. The minimum number of samples to be collected each year for veterinary drug residue analysis must be at least 1 per 100 tonnes of annual production.

Food contaminants are substances that may be present in fish and fishery products due to environmental contamination, cultivation practices or production processes. If present above certain levels, these substances can pose a threat to human health. Regulations of the European Union (Member Organization) ensure that food placed on the market is safe to eat and does not contain contaminants at levels that could threaten human health. Maximum levels for certain contaminants in fishery products are set in Regulation EC/1881/2006 (EC, 2006a). This regulation includes MRLs for heavy metals such as lead, cadmium and mercury and for dioxins and PCBs and polycyclic aromatic hydrocarbons. Methods for sampling and analysing fish for the control of levels of lead, cadmium, mercury and benzo- α -pyrene are included in Regulation EC/333/2007 (EC, 2007b) and for dioxin and dioxin-like PCBs in Regulation EC/1883/2006 (EC, 2006b).

7.2.17 Inspections and auditing to verify compliance

The European Commission has three main instruments at its disposal to ensure that legislation of the European Union (Member Organization) is properly implemented and enforced. It verifies the transposition by member States of legislation of the European Union (Member Organization) into national laws, and it analyses reports received from member States and third countries on the application of aspects of legislation, such as national residue programmes and animal feed controls. In addition, it carries out inspections in member States and third countries to check the implementation and enforcement of legislation of the European Union (Member Organization) by national competent authorities.

The control function at the level of the European Union (Member Organization) is mainly the responsibility of the Food and Veterinary Office (FVO),⁵⁶ a directorate of DG Health and Consumers. Its main task is to carry out on-the-spot inspections to evaluate national control systems, to report on its findings and to follow up on the action taken by national competent authorities in response to its reports. The European Commission has published guidance for the importation of fish and fishery products from third countries.⁵⁷

7.2.18 Conclusion

The integrated approach of the European Union (Member Organization) to food safety aims to ensure a high level of food safety, animal health, animal welfare and plant health within the European Union (Member Organization) through coherent

⁵⁵ http://ec.europa.eu/food/food/chemicalsafety/residues/sampling_levels_frequencies_jme.doc

⁵⁶ http://ec.europa.eu/food/fvo/index_en.cfm

⁵⁷ http://ec.europa.eu/food/international/trade/im_cond_fish_en.pdf

farm-to-fork measures and adequate monitoring, while ensuring the effective functioning of the internal market. Regulations, directives and decisions in the food safety control area are regularly updated and published by the European Commission on its website.⁵⁸

7.3 THE UNITED STATES OF AMERICA (TIM HANSEN)

7.3.1 The structure of regulatory authority in the United States of America

The United States of America has a decentralized system for food safety and quality regulation. There are no fewer than 17 federal government agencies involved in food regulation. The two most important agencies are the Food and Drug Administration (FDA) of the Department of Health and Human Services, which regulates all food except meat and poultry, and the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA), which is primarily responsible for meat and poultry. The Environmental Protection Agency (EPA) regulates the safety of water. The Agricultural Marketing Service (AMS) offers product quality and grading services for a fee to all food commodity groups except seafood. Seafood quality and safety services for a fee are provided by the Seafood Inspection Program of National Oceanic and Atmospheric Administration (NOAA) Fisheries within the Department of Commerce. The Department of Homeland Security is involved in ensuring that intentional product adulteration does not occur.

7.3.1.1 Food and Drug Administration

The FDA exercises regulatory control over most food, drugs, biologics and medical devices. It derives its authority from the Federal Food Drug and Cosmetic Act, the Public Health Act and the Bioterrorism Act. Upon entry to the United States of America, all imported food products are subject to inspection and possible detention or refusal of entry. The FDA also monitors the domestic food supply through a system of laboratory analysis of randomly selected food products. More importantly, the FDA performs regulatory inspections of all food establishments to determine whether the conditions of manufacturing are sufficiently controlled to prevent food safety hazards.

7.3.1.2 United States Department of Agriculture

Food Safety Inspection Service: The FSIS of the USDA has regulatory control over meat and poultry consumed in the United States of America. It derives its authority through the Federal Meat Inspection Act, the Poultry Products Inspection Act and the Egg Products Inspection Act. Any “amenable species”, which includes beef, pork, sheep, chickens and turkeys, covered under these acts is closely regulated through an in-plant inspection presence at least at the slaughter operation. The animals undergo an anti-mortem health evaluation and their carcasses a post-mortem evaluation for food safety.

Agricultural Marketing Service (AMS): The AMS of the USDA offers product grading and quality services on a fee-for-service basis to the food industry. It has programmes in most food areas including meat grading, fruits and vegetables, milk and dairy and processed products. The AMS does not normally evaluate firms and products for food safety but relies on the FDA or FSIS to perform these functions.

7.3.1.3 Environmental Protection Agency

The EPA is a stand-alone cabinet-level organization of the United States Federal Government. Its director is a political appointee who reports directly to the President. The EPA regulates water safety and pesticide residues in foods. It is authorized to do so under the Clean Water Act and the Federal Food, Drug and Cosmetic Act.

⁵⁸ http://ec.europa.eu/food/index_en.htm

7.3.1.4 *Department of Homeland Security*

Customs and Border Protection: Customs and Border Protection (CBP) works to prevent terrorist acts, including intentional adulteration of food shipped to the United States of America. It also monitors imports to ensure that they are legitimate goods and that appropriate duties are paid.

7.3.1.5 *Seafood Inspection Program of NOAA Fisheries, Department of Commerce*

The Seafood Inspection Program (SIP) offers a variety of services on a voluntary fee-for-service basis to the seafood industry both domestically and internationally. These services include product grading and quality evaluation, auditory oversight of HACCP programmes to ensure compliance to FDA laws and regulations and to allow the SIP to rely on the results of the firm's system of control to issue certificates and grade marks. The SIP also ensures that contractual firms adhere to all appropriate laws and regulations, including labelling, sanitation and process controls. This programme inspected about 2.1 billion pounds (almost 1 million tonnes) of seafood in 2010, about 41 percent of the total consumption in the United States of America.

In 2009, the SIP and FDA signed a memorandum of understanding that better defined the working relationship between the two agencies in the area of seafood regulation.⁵⁹

7.3.1.6 *State regulation*

Most state governments have food safety laws that are applicable to seafood products. These are usually general food safety, food handling and sanitation requirements although some states do require the implementation of HACCP systems. State food safety officials tend to pay more attention to economic factors than do federal regulators, e.g. correct labelling, net weights, breeding percentages. Two states, Alaska and Maine, have mandatory seafood inspection programmes. These states export large amounts of wild-caught seafood and want to ensure that the product is acceptable to consumers in other states and countries.

7.3.2 *Important laws, regulations and guidance*

7.3.2.1 *Food, Drug and Cosmetic Act*

This law⁶⁰ covers all food (except meat and poultry), drugs and cosmetics.

7.3.2.2 *Public Health Act*

This act⁶¹ is a compendium of laws that promote public health.

7.3.2.3 *Agricultural Marketing Act*

The act⁶² provided for voluntary grading programmes for all food commodities under the AMS that promoted the safety and quality of food.

7.3.2.3 *Fish and Wildlife Act*

This act⁶³ transferred seafood inspection from the USDA to the Department of the Interior (DOI) Bureau of Commercial Fisheries (later the NOAA). It also gave the DOI the authority to perform food safety inspections.

⁵⁹ www.seafood.nmfs.noaa.gov/2009_FDA-NOAA_MOU_seafood%20inspection.pdf

⁶⁰ www.fda.gov/opacom/laws/fdcact/fdctoc.htm

⁶¹ www.fda.gov

⁶² http://edocket.access.gpo.gov/cfr_2008/janqtr/pdf/7cfr53.1.pdf

⁶³ www.fws.gov/laws/lawsdigest/FWACT.HTML

7.3.2.5 *Bioterrorism Act 2002*

This act⁶⁴ calls for security measures for food, drugs and drinking-water and national preparedness for terrorist acts.

7.3.2.6 *Lacey Act*

This act⁶⁵ is designed to protect wildlife from illegal exploitation. It allows any federal or state law to be used as a basis of prosecution. It is useful to fisheries enforcement officers and food and drug officers in taking legal action against illegally caught or misbranded wild seafood.

7.3.2.7 *Food Safety Modernization Act*

This newly enacted law is designed to better enable the FDA to enforce food safety and better protect public health. See a complete description of this law below (Section 7.3.4.3).

7.3.2.8 *Country of Origin Labeling Act*

The following explanation may be found at the website operated by the AMS.⁶⁶

“Country of Origin Labeling (COOL) is a labeling law that requires retailers, such as full-line grocery stores, supermarkets and club warehouse stores, notify their customers with information regarding the source of certain foods. Food products (covered commodities) contained in the law include muscle cut and ground meats: beef, veal, pork, lamb, goat, and chicken; wild and farm-raised fish and shellfish; fresh and frozen fruits and vegetables; peanuts, pecans, and macadamia nuts; and ginseng. Regulations for fish and shellfish covered commodities (7 CFR Part 60) became effective in 2005. The final rule for all covered commodities (7 CFR Part 60 and Part 65) went into effect on March 16, 2009. AMS is responsible for administration and enforcement of COOL.”

The COOL law requires that:

“Under this final rule, a fish or shellfish imported covered commodity shall retain its origin as declared to CBP at the time the product enters the United States, through retail sale, provided it has not undergone a substantial transformation (as established by CBP [Custom Border Protection]) in the United States.”

“[W]ild fish and shellfish, if a covered commodity was imported from country X and substantially transformed (as established by CBP) in the United States or aboard a United States flagged vessel, the product shall be labelled at retail as “From [country X], processed in the United States.” Alternatively, the product may be labelled as “Product of country X and the United States”. The covered commodity must also be labelled to indicate that it was derived from wild fish or shellfish. In the case of farm-raised fish, if a covered commodity was imported from country X at any stage of production and substantially transformed (as established by CBP) in the United States, the product shall be labelled at retail as “From [country X], processed in the United States.” Alternatively, the product may be labelled as “Product of country X and the United States”. The covered commodity shall also be labelled to indicate that it was derived from farm-raised fish or shellfish.”

7.3.2.9 *21 Code of Federal Regulation 110*

This regulation⁶⁷ specifies GMPs for food production.

⁶⁴ www.fda.gov/regulatoryinformation/legislation/ucm148797.htm

⁶⁵ www.aphis.usda.gov/plant_health/lacey_act/

⁶⁶ www.ams.usda.gov/AMSV1.0/COOL

⁶⁷ www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=110

7.3.2.10 21 Code of Federal Regulation 113

This regulation⁶⁸ addresses low-acid canned food requirements.

7.3.2.11 21 Code of Federal Regulation 123

This regulation⁶⁹ is the seafood HACCP requirements for all seafood produced or shipped to the United States of America.

7.3.2.12 50 Code of Federal Regulation 260

This is the regulation⁷⁰ covering inspection and certification of establishments and fishery products for human consumption.

7.3.2.13 The Fish and Fishery Products Hazard Guide

In April 2011, the FDA published the fourth edition of the Fish and Fishery Product Hazard Guide. This guidance is intended to advise the seafood industry on how to conduct a hazard analysis, and develop a suitable preventive HACCP plan that will satisfy regulators and ensure food safety. The guide has chapters describing all the major known food safety hazards for seafood including process-related hazards and species-related hazards. This publication is essential in applying the HACCP concept and regulatory compliance. As there is no other publication that has the scope and detail to design and implement an adequate HACCP plan, it is recognized worldwide as the best source of information for this complex and difficult subject.

7.3.3 Food law implementation for seafood

7.3.3.1 Domestic implementation for seafood

Currently, the two federal agencies that regulate the product and conditions of production are the FDA and the NOAA Fisheries Seafood Inspection Program. The FDA focuses its inspection effort on the conditions of production that may affect the safety of the product, e.g. sanitation and preventive HACCP programmes. Investigators from the FDA take samples of seafood on a routine basis for analysis for any possible hazard that may occur in that product. The SIP concentrates on ensuring compliance with FDA laws and regulations and also evaluates product for safety and quality.

The two most important regulations for seafood are the current GMPs 21 Code of Federal Regulation 110 and the seafood HACCP Regulation 21 Code of Federal Regulation 123. The current GMPs deal mainly with sanitation, food handling and hygiene. These requirements are applicable to all food products. These are the so-called prerequisite programmes for preventive control systems that are the basic tenet of any food safety system. The seafood HACCP regulations are specific to seafood and require that appropriate preventive controls of likely hazards are established for the processing of all seafood products. A system of systems verification including records review is also required to ensure that the system is working properly.

This regulation is supported by the Fish and Fishery Products Hazard Guide, which gives detailed instruction about how to identify hazards, write and implement a HACCP plan and other regulatory requirements that seafood producers need to be aware of.

Inspections by the FDA are auditory in nature. Inspectors will visit a plant unannounced and evaluate its sanitation conditions and HACCP systems. These inspections will generally take 1–5 days to complete. When the investigator has completed the inspection, a so-called form 483 will be issued that lists objectionable

⁶⁸ www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=113

⁶⁹ www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=123

⁷⁰ www.seafood.nmfs.noaa.gov/50CFR260-261.PDF

observations. The investigator will usually advise the firm to submit a written description of how it intends to correct the problems.

It is advisable that the firm responds immediately to the observations and submits appropriate corrections. In many cases, responsiveness by the firm will convince FDA officials that further regulatory action is unnecessary. If the firm believes that the FDA investigator's observations are incorrect or not scientifically based, it should inform the FDA in writing of its reasoning. The FDA has a policy related to HACCP controls that it has called the "continuation policy", which states that the firm may petition the FDA if it believes that its system of control has a sound scientific basis but does not conform to the Fish and Fishery Product Guide. If the reasoning appears to be valid, scientists at the Center for Food Safety and Applied Nutrition will evaluate the information submitted by the firm for scientific validity. If the firm's reasoning is acceptable, no further regulatory actions are likely to take place for that issue.

The FDA also requires that all food manufacturers register under the Bioterrorism Act of 2002. The process is accomplished by filling out a web-based form and submitting the information to the FDA.

The SIP oversees about one-third of the United States consumption of seafood. It has contracts with many of the larger firms in the United States of America and, depending on the type of service, it will be in the plant and inspect on a continuous basis or will audit the firm four or more times per year. In either case, firms will undergo a rigorous systems audit for preventive controls and sanitation at least four times per year. The SIP will also require that a firm submit written corrective actions to systems audit checklists. If corrections are not made, the contract may be suspended or revoked and the firm will not receive the certifications and grade marks that its customers require.

7.3.3.2 Import implementation for seafood

Imported seafood is subject to the regulatory oversight of the FDA. Any consignment offered for entry into the United States of America is subject to inspection by FDA import officers. These officers use a digital system for selection of seafood products that is based on the relative risk of the product to the consumer. Theoretically, a cooked RTE product should be sampled and analysed at a much higher rate than raw products with no inherent hazards. Once a consignment is targeted for inspection and analysis, it may be subject to a visual examination or more rigorous analytical testing for contaminants. If the officer sees any discrepancy with the product that constitutes an "appearance of adulteration", the importer then assumes the burden of proof that the product is not adulterated and it may be tested at the expense of the importer or denied entry. In any case, the product will be placed in expensive bonded warehouse until the matter is resolved. An appearance can be mislabelling, inadequate packaging protecting the product or anything that seems to be non-compliant with the regulations and laws. If contaminants are found and there is a reasonable way to eliminate them, e.g. cooking raw product with microbiological contamination, then the importer may petition the FDA to do so with specific explanations about how the processing will eliminate the hazard.

If the FDA believes that product imported from a particular firm, country or region has a high probability of adulteration, it may issue an import alert. An import alert will list all the affected firms, countries or regions and it will require appropriate analytical testing on each lot offered for importation into commerce of the United States of America. Firms, countries or regions will have to show that the root cause of the problem that created the adulteration has been eliminated. For seafood firms that are subject to the Seafood HACCP Regulation, this usually requires that the FDA or a reliable third party has verified that the correction has occurred. This may cause

problems if there are many affected firms as it may take the FDA a significant period to verify the corrections.

Importers must give prior notice to the CBP that a shipment is going to be offered for entry under the food protection provisions of the Bioterrorism Act. The time limitations vary according to what conveyance the product is transported in.

Importers also must comply with “21 Code of Federal Regulation 123.12 Special requirements for imported products”. The purpose of this provision in the HACCP regulations is to ensure that products entering into United States commerce are in compliance with the Seafood HACCP Regulation similar to domestically produced seafood. The importer of record must buy seafood from a country with an active memorandum of understanding with the FDA or have written verification procedures that outline product food safety specifications and affirmative steps as follows:

- obtaining from the foreign processor the HACCP and sanitation monitoring records required by this part that relate to the specific lot of fish or fishery products being offered for import;
- obtaining either a continuing or lot-by-lot certificate from an appropriate foreign government inspection authority or competent third party certifying that the imported fish or fishery product is or was processed in accordance with the requirements of this part;
- regularly inspecting the foreign processor’s facilities to ensure that the imported fish or fishery product is being processed in accordance with the requirements of this part;
- maintaining on file a copy, in English, of the foreign processor’s HACCP plan, and a written guarantee from the foreign processor that the imported fish or fishery product is processed in accordance with the requirements of this part;
- periodically testing the imported fish or fishery product, and maintaining on file a copy, in English, of a written guarantee from the foreign processor that the imported fish or fishery product is processed in accordance with the requirements of this part or other such verification measures as appropriate that provide an equivalent level of assurance of compliance with the requirements of this part;
- an importer may hire a competent third party to assist with or perform any or all of the verification activities specified above, including writing the importer’s verification procedures on the importer’s behalf.

7.3.3.3 *Monitoring and analysis for seafood*

The FDA does not perform a large volume of analytical monitoring for domestic product. The Center for Food Safety and Applied Nutrition has an annual compliance plan that specifies, among other inspection activities, what products will be sampled and what analysis will occur. There is also a standing sampling plan called Toxic Elements, where appropriate chemical analysis is performed at a specified rate.

Imported products are more likely to be monitored and analysed than domestic products although the overall monitoring rate is about one percent. Import officers use a digital risk assessment system to make random choices of consignment for sampling, and the appropriate analysis is then performed.

7.3.3.4 *Regulatory actions for seafood*

During routine inspections of seafood manufacturing facilities, FDA investigators may find conditions of production or lack of preventive controls that they judge to be serious or critical in nature. The investigator will note the egregious condition on Form 483 List of Observations. If the firm does not correct the deficiency, the FDA will issue a Warning Letter. This is an official letter informing the firm that the FDA intends to take regulatory action through the court system. If the FDA finds similar

conditions on a follow-up inspection, regulatory action will probably occur. This will mean that the FDA will pursue a court action. However, the agency must go through an exhaustive review process before the court action can go forwards. This will include a review of the sufficiency of the evidence by the district that will classify the action and send the case file to the Center for Food Safety and Applied Nutrition, which will again look at the evidence development through the Office of Compliance and send it to the Office of Food Safety Division of Seafood for scientific review. If the investigator and district scientific reasoning is sound, the case file goes to the Office of General Counsel for final legal review. Once the Office of General Counsel is satisfied that a sufficient case exists, the assigned attorney will refer the case to the United States Attorney (who works for the Department of Justice) near the location of the manufacturing plant who may or may not choose to prosecute the case. If the prosecution is successful, the federal court will generally issue an injunction against the firm, which is an order by the court to stop all processing until the FDA is satisfied that the egregious conditions are corrected. As this is an elaborate process, only a few regulatory actions are adjudicated in court each year.

If the FDA has knowledge that a lot of food is adulterated, it may take action against the product itself and seek a seizure of the product by federal officials. As the FDA does not do a great deal of product inspection for domestic seafood, this is generally a rare event. However, imports are routinely analysed for appropriate hazards. If an imported consignment is found to be adulterated, it can be reprocessed to eliminate the hazard (if possible), destroyed or not allowed in commerce and shipped out of the United States of America.

7.3.4 Recent changes in seafood regulation

7.3.4.1 Proposed regulatory changes for catfish

The 2008 Farm Bill granted the FSIS regulatory control over catfish. It amended the Federal Meat Inspection Act (FMIA) to include catfish as an “amenable species” similar to beef, pork and lamb. It requires that a federal inspector be present during the slaughter operation and that food safety preventive controls be in place as well as controls for labelling and marks. The 2002 Farm Bill defined catfish as North American catfish in the Ictaluridae family. The 2008 Farm Bill allows the Secretary of Agriculture to define the scope of the catfish regulation. That decision has yet to be made although Congress mandated that it be decided by early 2009. The new definition may be as broad as the order *Siluriformes* which encompasses all catfish including *Pangasius* or limited to *Ictaluridae*.

The FMIA also requires that imports be produced under equivalent controls to the United States of America before the product can be allowed into the United States market. As equivalent controls do not currently exist in catfish-producing countries, this provision would effectively stop importation until such a system was established by the exporting country’s competent authority and audited and verified by the FSIS. This process typically takes two to three years or longer.

The 2008 Farm Bill legislation also allows the Secretary of Agriculture to expand regulation to all farm-raised fishery products if so petitioned by the interested public. If such a decision were made, this could include other commodity areas such as shrimp, tilapia and other cultured species. These species would then also be subject to import restrictions until an equivalent level of control was established by the exporting country.

This legislation, if enacted, has the potential for a significant effect on the regulation of aquaculture products for food safety and the ability for exporting nations to penetrate the United States market with the species concerned.

As of summer 2013, the FSIS has not implemented this legislation nor has the Secretary of Agriculture decided the scope of the USDA regulatory oversight. It

appears unlikely that implementation will occur. The United States Government is experiencing significant budgetary problems and new and duplicative federal programmes will be targets for eliminating wasteful spending. Moreover, the FSIS did not receive funding for this programme in the Fiscal Year 2012 budget. It is estimated that it would cost about US\$160 million to implement fully. This money will have to come from the meat and poultry inspection budget, which is also underfunded. As important food safety controls will be affected, it does not appear likely that there will be any funding for catfish regulatory programmes. In spring 2013, legislation was proposed to repeal the amendment to the FMIA.

7.3.4.2 Cross-cutting food safety activities in the United States Federal Government

Since mid-2007, there have been several food safety incidents that have caused considerable public concern. These incidents have generated interest from the federal government. In summer 2007, the Office of Management and Budget, the executive branch of the White House, asked the various agencies to form an ad hoc group to examine the safety problems of imports and what measures the United States Government might take to ensure safety to the American consumer. This group also looked at food safety measures as well as toys and other commodities. The FDA also published the Food Protection Plan⁷¹ that outlined its plan to better protect the consumer from food safety risks. At this time, several legislative acts were proposed that would enhance food safety in the United States of America. Because of the concern over food safety risks, it is likely that food regulation, including seafood, will change significantly in the years ahead. The authorizing of the FSIS to regulate catfish is perhaps the first example of this change. Anyone selling seafood to customers in the United States of America should pay careful attention to regulatory requirements.

7.3.4.3 Food Safety Modernization Act Public Law 111-353 21 USC 2201

In January 2011, the President of the United States of America, Barack Obama, signed the Food Safety Modernization Act (FSMA) into law. This is a significant new law that enables the FDA to implement much stronger enforcement of food safety measures to better protect public health. This will include new regulatory tools and enforcement authorities.

The FDA website for the act⁷² has the following description of the new act. The following are among the FDA's key new authorities and mandates. Specific implementation dates specified in the law are noted in parentheses:

“Prevention: For the first time, FDA will have a legislative mandate to require comprehensive, science-based preventive controls across the food supply. This mandate includes:

- **Mandatory preventive controls for food facilities:** Food facilities are required to implement a written preventive controls plan. This involves: (1) evaluating the hazards that could affect food safety, (2) specifying what preventive steps, or controls, will be put in place to significantly minimize or prevent the hazards, (3) specifying how the facility will monitor these controls to ensure they are working, (4) maintaining routine records of the monitoring, and (5) specifying what actions the facility will take to correct problems that arise. (Final rule due 18 months following enactment)
- **Mandatory produce safety standards:** FDA must establish science-based, minimum standards for the safe production and harvesting of fruits and vegetables. Those standards must consider naturally occurring hazards, as well as those that may be introduced either unintentionally or intentionally, and

⁷¹ www.fda.gov/Food/GuidanceRegulation/FoodProtectionPlan2007/default.htm

⁷² www.fda.gov/Food/GuidanceRegulation/FSMA/ucm239907.htm

must address soil amendments (materials added to the soil such as compost), hygiene, packaging, temperature controls, animals in the growing area and water. (Final regulation due about 2 years following enactment)

- Authority to prevent intentional contamination: FDA must issue regulations to protect against the intentional adulteration of food, including the establishment of science-based mitigation strategies to prepare and protect the food supply chain at specific vulnerable points. (Final rule due 18 months following enactment).

Inspection and Compliance: The FSMA recognizes that preventive control standards improve food safety only to the extent that producers and processors comply with them. Therefore, it will be necessary for FDA to provide oversight, ensure compliance with requirements and respond effectively when problems emerge. FSMA provides FDA with important new tools for inspection and compliance, including:

- Mandated inspection frequency: The FSMA establishes a mandated inspection frequency, based on risk, for food facilities and requires the frequency of inspection to increase immediately. All high-risk domestic facilities must be inspected within five years of enactment and no less than every three years thereafter. Within one year of enactment, the law directs FDA to inspect at least 600 foreign facilities and double those inspections every year for the next five years.
- Records access: FDA will have access to records, including industry food safety plans and the records firms will be required to keep documenting implementation of their plans.
- Testing by accredited laboratories: The FSMA requires certain food testing to be carried out by accredited laboratories and directs FDA to establish a program for laboratory accreditation to ensure that United States food testing laboratories meet high quality standards. (Establishment of accreditation program due 2 years after enactment).

Response: The FSMA recognizes that FDA must have the tools to respond effectively when problems emerge despite preventive controls. New authorities include:

- Mandatory recall: The FSMA provides FDA with authority to issue a mandatory recall when a company fails to voluntarily recall unsafe food after being asked to by FDA.
- Expanded administrative detention: The FSMA provides FDA with a more flexible standard for administratively detaining products that are potentially in violation of the law (administrative detention is the procedure FDA uses to keep suspect food from being moved).
- Suspension of registration: FDA can suspend registration of a facility if it determines that the food poses a reasonable probability of serious adverse health consequences or death. A facility that is under suspension is prohibited from distributing food. (Effective 6 months after enactment)
- Enhanced product tracing abilities: FDA is directed to establish a system that will enhance its ability to track and trace both domestic and imported foods. In addition, FDA is directed to establish pilot projects to explore and evaluate methods to rapidly and effectively identify recipients of food to prevent or control a food-borne illness outbreak. (Implementation of pilots due 9 months after enactment)
- Additional Recordkeeping for High Risk Foods: FDA is directed to issue proposed rulemaking to establish recordkeeping requirements for facilities that manufacture, process, pack, or hold foods that the Secretary designates as high-risk foods. (Implementation due 2 years after enactment).

Imports: The FSMA gives FDA unprecedented authority to better ensure that imported products meet United States standards and are safe for United States consumers. New authorities include:

- **Importer accountability:** For the first time, importers have an explicit responsibility to verify that their foreign suppliers have adequate preventive controls in place to ensure that the food they produce is safe. (Final regulation and guidance due 1 year following enactment)
- **Third Party Certification:** The FSMA establishes a program through which qualified third parties can certify that foreign food facilities comply with United States food safety standards. This certification may be used to facilitate the entry of imports. (Establishment of a system for FDA to recognize accreditation bodies is due 2 years after enactment)
- **Certification for high risk foods:** FDA has the authority to require that high-risk imported foods be accompanied by a credible third party certification or other assurance of compliance as a condition of entry into the United States of America
- **Voluntary qualified importer program:** FDA must establish a voluntary program for importers that provides for expedited review and entry of foods from participating importers. Eligibility is limited to, among other things, importers offering food from certified facilities. (Implementation due 18 months after enactment)
- **Authority to deny entry:** FDA can refuse entry into the United States of America of food from a foreign facility if FDA is denied access by the facility or the country in which the facility is located.

Enhanced Partnerships: The FSMA builds a formal system of collaboration with other government agencies, both domestic and foreign. In doing so, the statute explicitly recognizes that all food safety agencies need to work together in an integrated way to achieve our public health goals. The following are examples of enhanced collaboration:

- **State and local capacity building:** FDA must develop and implement strategies to leverage and enhance the food safety and defence capacities of State and local agencies. The FSMA provides FDA with a new multi-year grant mechanism to facilitate investment in State capacity to more efficiently achieve national food safety goals.
- **Foreign capacity building:** The law directs FDA to develop a comprehensive plan to expand the capacity of foreign governments and their industries. One component of the plan is to address training of foreign governments and food producers on United States food safety requirements.
- **Reliance on inspections by other agencies:** FDA is explicitly authorized to rely on inspections of other Federal, State and local agencies to meet its increased inspection mandate for domestic facilities. The FSMA also allows FDA to enter into interagency agreements to leverage resources with respect to the inspection of seafood facilities, both domestic and foreign, as well as seafood imports.

Additional partnerships are required to develop and implement a national agriculture and food defence strategy, to establish an integrated consortium of laboratory networks, and to improve food-borne illness surveillance.”

There are several other food safety provisions that are not discussed or elaborated on the FDA website that will be important for all seafood producers who do business in the United States of America.

Authority to collect fees: For the first time, the FDA will be able to collect fees under the following circumstances:

- for any domestic re-inspection;
- for any domestic party that does not comply with a recall order;

- each importer participating in the voluntary qualified importer programme will be charged for administrative fees;
- importers subject to re-inspection.

The tentative rate for these fees will be US\$224 per hour, which will probably have a significant economic impact on the seafood industry.

Sanitary transportation of food: The FDA is directed by the United States Congress to enact regulations covering the safe transportation of food and to conduct a study of transportation of food safety related to transportation.

Laboratory accreditation and integrated consortium of laboratory networks: The United States Congress has directed the FDA to establish a programme for testing food by accredited laboratories and to create an available registry of accredited bodies and laboratories recognized by the accredited bodies.

Voluntary qualified importer programme: Section 302 of the FSMA provides for the establishment of a programme to provide for expedited review and importation of food offered for importation by importers that have voluntarily agreed to participate. This programme will be entirely voluntary. Interested parties must submit an application to the Secretary of Health and Human Services. The applicant's eligibility will be determined by the Secretary based on the following factors: known safety risks of the food, compliance history of the foreign supplier, capability of the regulatory system of the country of export, compliance of the importer to United States food safety standards, the record-keeping, testing, inspections and audits of facilities, traceability of articles of food, temperature controls, and sourcing practices of the importer and, finally, any other factor the Secretary determines appropriate.

Participants in this programme should be able to bring food products into United States commerce faster than those importers that do not participate and opt for the traditional import procedures.

Authority to require import certifications for food: Section 303 of the FSMA empowers the FDA to require that imported food be accompanied by a shipment specific certification or other assurance that the food meets applicable requirements of the FSMA. The FDA may require certification if the Secretary believes that the food is not compliant to FSMA or other food safety laws after assessing the food safety programmes, systems, and standards in the foreign country or region where the consignment is manufactured.

Inspection of foreign food facilities: Section 306 directs the Secretary of Health and Human Services to direct resources to inspection of foreign facilities, suppliers and food types that represent a high risk to ensure food safety and security of the United States food supply. Any foreign firm that refuses an inspection request by the FDA shall have all import consignments refused entry into commerce of the United States of America.

7.3.4.4 *Possible impact of the FSMA on the seafood industry*

Implementing this legislation will be a major undertaking for the FDA. It will probably take many years to complete. The more immediate concern is funding. The resources required for implementation will be considerable. At the same time, the United States Congress is concerned about recent increases in the budget deficit, so the needed funding may not be available. One way the FDA could generate funds is through the collection of fees. The FDA will have the ability to collect fees for many non-routine activities such as re-inspections, detentions, analytical tests and foreign inspections. In general, industry can expect:

- more cost due to fee collection;
- more requirements, e.g. regulation of transportation and HACCP for all firms;
- more detentions owing to increased scrutiny of imports;
- more facility inspections owing to mandated inspection frequencies;
- more testing as analytical capacity is increased.

7.3.5 Summary of the regulatory climate in the United States of America

The authority for seafood regulation in the United States of America is distributed among several federal agencies. This has resulted in a patchwork of regulatory oversight that has not always been as effective as it could be in ensuring safe seafood to the consumer. The decision by the United States Congress to grant authority for seafood COOL to the AMS and catfish regulation to the FSIS has furthered this trend. However, in recent years, regulatory control has been strengthened by better cooperation among the various agencies and by the FSMA of 2011. The FDA and the NOAA Seafood Inspection Program have been working more closely on seafood issues through a revamped memorandum of understanding that was completed in 2009. The FSMA will greatly increase the FDA's ability to strongly regulate all food and seafood. The recent mood in the United States Congress is to focus on reducing the overall debt of the United States Government, which will probably reduce the resources available to all seafood regulatory in the immediate future. It remains to be seen whether the United States Congress will grant the budget to increase the regulatory control of foods despite new, stronger regulatory food laws.

7.4 JAPAN (HAJIME TOYOFUKU)

7.4.1 Introduction

In Japan, the administration of food safety is based on the Food Safety Basic Law (enacted in May 2003), the Food Sanitation Law, the Abattoir Law, the Poultry Slaughtering Business Control and Poultry Inspection Law and other related laws.

There has been growing concern and distrust of regulatory food safety among the Japanese public, triggered by various problems, including the occurrence of bovine spongiform encephalopathy (BSE) in 2001. Against this background, Japan enacted the Food Safety Basic Law, a comprehensive law to ensure food safety to protect the health of the public. In the wake of the development of the basic law and other related laws, Japan has introduced a risk analysis approach to the national food safety control programme.

The approach is to scientifically assess risks (expressed as the probability and degree of adverse health effects) and identify and implement risk management options based on the outcomes of the risk assessment. The Food Safety Basic Law is responsible for the risk assessment, and the Food Sanitation Law and other related laws are responsible for risk management. The risk assessment is, in practice, conducted by the Food Safety Commission established under the Food Safety Basic Law.

The Food Sanitation Law covers two major responsibilities:

- The establishment of standards and specifications for food, food additives, equipment and food containers/packages, standards for food establishments and GHP, and specific manufacturing standards for certain foods;
- Inspections to see whether these established standards are met; the hygiene control programme from primary production to the retail sale of food; business licences, and advice to food-related businesses.

Health departments of local governments are mainly responsible for domestically produced food. In contrast, the border inspection for imported food is conducted by the central government (Ministry of Health, Labour and Welfare). The purpose of the law is to prevent health hazards arising from consumption of food, by making necessary regulations and taking any measures to protect public health.

7.4.2 Setting standards and specifications

Food safety must principally be ensured by a more preventive approach, such as product and process design and the application of GHPs and GMPs. In addition, under the Food Sanitation Law to ensure public health (MHLW, 1959), the Minister of Health, Labour and Welfare established the specifications and standards, including

microbiological criteria and standards for manufacturing methods, processing, preparing or preserving food, or for food additives intended for sale, and specifications for food utensils or containers/packages for sale or for use in business. Once it is recognized that a food and or food additive is not compliant with the specification and standards, the sale, distribution, import, use, preparation, and/or holding of the food is prohibited. Specifications and standards have been established for seafood categories indicated below (Article 11 of the Food Sanitation Law – Standards and specifications for food in general and specific foods). In fish and fisheries products, the specific standards and specifications are listed in Table 85.

TABLE 85
Seafood-related standard and specifications

Food categories	Standards and specifications	Description
Surimi products	Specifications	Regarding surimi products (except for uncooked surimi as a raw ingredient for cooked surimi products), coliforms shall be negative. For fish sausage and fish ham, the level of sodium nitrite should be not more than 0.05 g/kg (residual level as NO ₂).
	Standards of manufacturing	The process standard for cooking is that the centre part should reach more than 75 °C, or equivalent, and for fish sausage and fish ham, the centre part should reach 80 °C for 45 min, or equivalent.
	Standard of storage	Below 10 °C (except for retorted products).
Ikura (salmon roe), sujiko (salmon roe still in its sac), and tarako (cod roe)	Specifications	Sodium nitrite in these foods shall be not more than 0.005 g/kg. There are no microbiological criteria for these food categories.
Boiled crab and octopus	Specifications of composition	For boiled, then chilled, crab and octopus, the only microbiological criterion is <i>Vibrio parahaemolyticus</i> – negative in a 25 g sample. For boiled, then frozen, crab and octopus, a standard plate count should be less than 100 000/g, coliforms should be negative and <i>Vibrio parahaemolyticus</i> negative in a 25 g sample.
	Standards of processing	The octopus used for processing must be fresh. The water used for the processing must be potable water, sterilized seawater or artificial seawater prepared using potable water. The crab must be cooked at the centre part to a temperature of more than 70 °C for 1 minute, or equivalent. After the crab and/or octopus have been boiled, they must be promptly and sufficiently cooled using potable water. After cooling, boiled octopus and crab must be packed and kept in clean and easily washable, impermeable, covered containers made of metal, synthetic resin, etc. or otherwise protected from contamination.
	Standard of storage	Boiled octopus/crabs shall be stored at a temperature below 10 °C. Frozen boiled octopus/crab shall be stored at temperatures below minus 15 °C.
Fresh fish and shellfish intended to be consumed raw	Specifications of composition	Microbiological criteria for fresh fish and shellfish for raw consumption are: <i>Vibrio parahaemolyticus</i> : < 100 MPN count/g, alkaline peptone broth, 37 °C, overnight, then TCBS plate, 37 °C, overnight.
	Standards of processing	Water used for processing shall be potable, or pasteurized seawater, or artificial seawater made from potable water. In cases where fish and shellfish that are used as raw materials are frozen, they shall be thawed in a clean environment or in a water tank using potable water, pasteurized seawater, or artificial seawater made from potable water, and the water should be changed frequently. Fish and shellfish, as raw materials, shall be sufficiently washed with potable water, pasteurized seawater, or artificial seawater made from potable water to remove anything that might contaminate the product. During processing, no synthetic chemical additive shall be used (except sodium hypochlorite).
	Standard of storage	Fresh fish and shellfish must be placed in clean and hygienic containers and stored below 10 °C.

TABLE 85 (continued)

Food categories	Standards and specifications	Description
Oysters intended to be consumed raw	Specifications of composition	<p><i>Vibrio parahaemolyticus</i>: < 100 MPN/g.</p> <p><i>E. coli</i>: < 230 MPN/100 g product EC broth 5 tube fermentation tube, 44.5 °C, 24 h.</p> <p>Standard plate count: < 50 000/g product (by plate count agar, 35 °C, 48 ± 3 h).</p>
	Standards of processing	<p>Oysters used as raw material must be collected from waters where the most probable number (MPN) of coliform bacteria is no more than 70 per 100 ml of seawater, or collected from other waters but cleaned using either seawater, where the MPN of coliforms is no more than 70/100 ml or artificial saltwater with a 3% salinity.</p> <p>The same microbiological quality of water should be used for temporary storage of the oysters.</p> <p>Oysters must be thoroughly washed in clean water after they are caught or landed.</p> <p>Oysters must be processed in a hygienic location.</p> <p>The apparatus used for shucking must be easy to wash and sterilizable. Before use, it must be cleaned and sterilized.</p>
	Standard of storage	Oysters must be packed in a clean and sanitary container and stored at a temperature of 10 °C or below for chilled products, and minus 15 °C or below for frozen products.
Frozen fish or shellfish intended to be consumed raw	Specifications of composition	<p>Standard plate count: < 100 000 /g product by plate count agar, 35 °C, 48 ± 3 h.</p> <p>Coliform: negative (means dark red colonies are not identified on the desoxycolate agar plates).</p> <p><i>Vibrio parahaemolyticus</i>: < 100 MPN count/g.</p>
	Standards of processing	<p>The water used for the processing must be potable water, sterilized seawater or artificial seawater prepared using potable water.</p> <p>In cases where fish and shellfish that are used as raw materials are frozen, they shall be thawed in a clean environment or in a water tank by using potable water, pasteurized seawater, or artificial seawater made from potable water, and the water should be changed frequently.</p> <p>Fish and shellfish that are used as raw materials shall be sufficiently washed with potable water, pasteurized seawater, or artificial seawater made from potable water to remove anything that might contaminate the product.</p> <p>During processing, no synthetic chemical additive shall be used (except sodium hypochlorite).</p> <p>Processed fish and shellfish for raw consumption shall be frozen immediately after processing.</p>
	Standard of storage	<p>Frozen product shall be stored at minus 15 °C or below.</p> <p>Frozen product shall be wrapped in clean and sanitary plastic, aluminium foil or waterproof processed paper for storage.</p>
Frozen food	Specifications of composition	<p>Regarding frozen food, there are three categories in the standard. The key factors to be considered when microbiological criteria are to be applied are: whether cooking is needed before consumption, and whether a cooking process is involved immediately before freezing.</p> <p>(a) served after cooking and cooked immediately before freezing: aerobic micro-organisms: 30 °C < 100 000, coliforms: not detectable;</p> <p>(b) served without cooking: aerobic micro-organisms: 30 °C < 100 000, coliforms: not detectable;</p> <p>(c) served after cooking and not cooked immediately before freezing: aerobic micro-organisms: 30 °C < 3 000 000, <i>Escherichia coli</i>: not detectable (gas not produced in any of 3 tubes of EC medium).</p>

7.4.3 Food additives

In Japan, only food additives designated by the Minister of Health, Labour and Welfare are allowed, and then only under strict usage standards. To export seafood to Japan, care is needed in the use of food additives, as the use of some food additives is controlled by strict conditions. For example, benzoic acid, a well-known preservative, can only be used for caviar, with the maximum concentration of 2.5 g/kg. The use of sulphur dioxide is limited to shrimp flesh and frozen crab meat, at a concentration of less than 0.1 g/kg. The use of sorbic acid is limited to cooked surimi products (not more than 2.0 g/kg), sea urchin (not more than 2.0 g/kg), smoked squid and octopus (not more than 1.5 g/kg) and dried fish and shellfish (not more than 1.0 g/kg).

In addition, artificial food colours are not permitted in raw fish and shellfish. Tertiary butylhydroquinone, azorubine and polysorbate are typical examples of food additives that are not permitted under the Food Sanitation Law.

7.4.4 Environmental contaminants and marine biotoxins

Provisional regulatory limits of environmental contaminants and natural toxins in fish and shellfish have been established.

Regarding PCBs, the provisional regulatory limit for fish and shellfish from pelagic or offshore waters is 0.5 ppm, while the limit for fish and shellfish from coastal waters or freshwater is 3 ppm.

Regarding mercury, provisional regulatory limits of total mercury and methylmercury were established in 1973 at 0.4 ppm and 0.3 ppm, respectively. However, these regulatory limits of mercury do not apply to tuna (tuna, swordfish and bonito), fish and shellfish from rivers and inland water areas and deep-sea fish and shellfish.

Regarding shellfish toxins, regulatory limits for PSP are set at less than 4 mouse units/g, (where 1 mouse unit is the amount of toxin required to kill a 20 g mouse in 15 min). The limit for DSP is less than 0.5 mouse units per gram. These are no regulatory limits established for other marine biotoxins.

7.4.5 Inspection of imported food at quarantine stations

The inspection system at quarantine stations for imported foods into Japan is illustrated in Figure 60. In principle, all food importers should submit import notifications to the Minister of Health, Labour and Welfare of Japan through quarantine offices upon arrival of the cargo (Article 26 of the Food Sanitation Law).

At the quarantine offices, the food safety inspectors examine the notification and attached documents in order to determine the necessity for on-site, organoleptic, chemical, physical and microbiological examinations. If the inspector does not recognize any potential violation of the Food Sanitation Law, e.g. there has been no past history of food safety hazards in the food, the inspector accepts the notification.

About 10 percent of any cargo is subject to monitoring tests, which are planned to monitor the prevalence and concentration of chemical residues, indicator micro-organisms and pathogens in food. If the notified food is under the category of 100 percent mandatory testing, the food will be examined to make sure it complies with the Food Sanitation Law and its standards and specifications, and it will be held in warehouses around the port of entry until the test result indicates that the food complies with the Food Sanitation Law and regulations.

If it is found that the notified food does not comply with the Food Sanitation Law, it must be shipped back to the country of origin or discarded.

The trends in import food notifications, the weight of imported food notified, the number of total analyses and the number of rejected cases are summarized in Table 86. The total notifications in the 2009 fiscal year were 1.82 million, a 19-fold increase compared with that of 1965 and a 4.7-fold increase compared with 1985. The total

weight in 2009 was 30.6 million tonnes, which was a 2.4-fold increase compared with that of 1965 and a 1.35-fold increase compared with 1985.

In 2009, 12.7 percent of notified imported foods were analysed, and 1 559 (0.086 percent of the notifications) were rejected due to violations of the Food Sanitation Law. Out of the total of 30.4 million tonnes of imported food, fish and fishery products accounted for 2.2 million tonnes (7.3 percent) (MHLW, 2010a). Data for 2010 and 2011 are also presented, and this shows drops in rejections from a larger number of notifications.

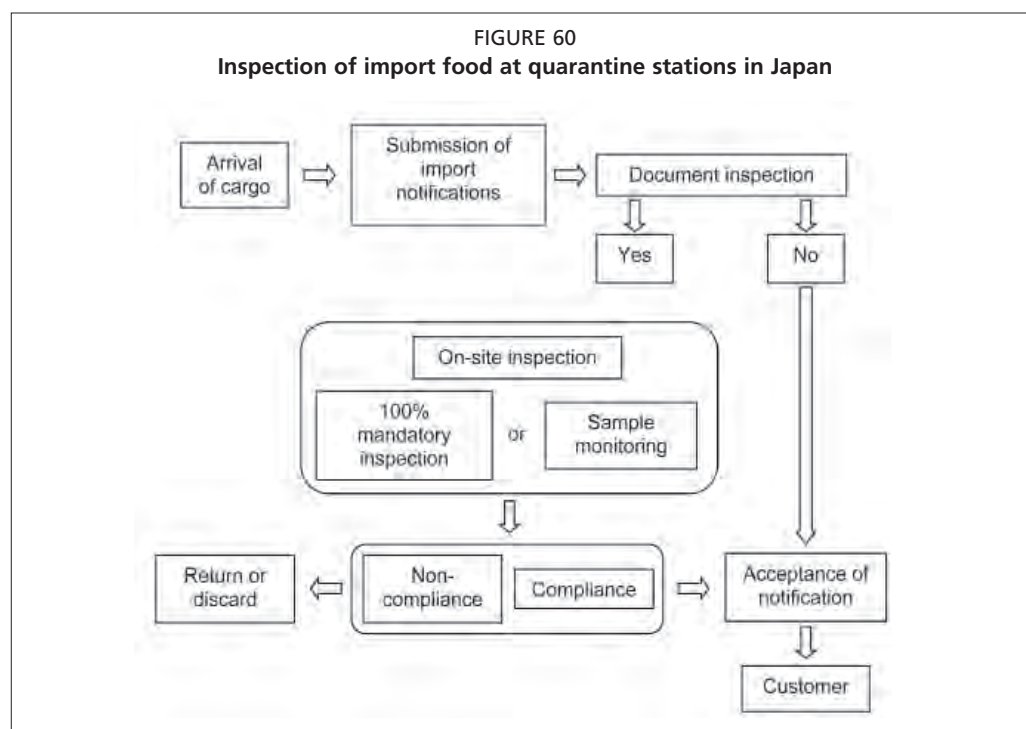


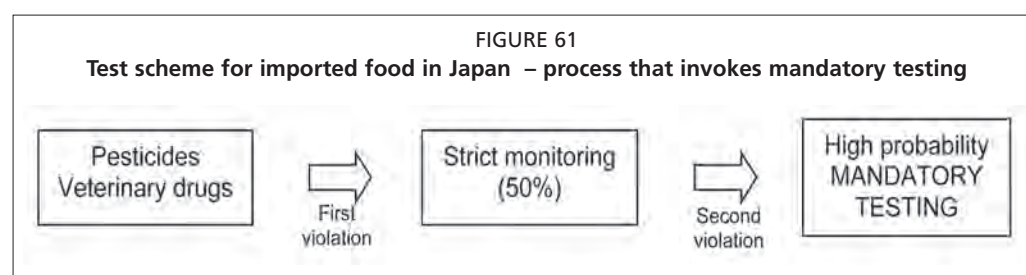
TABLE 86

Number of notifications, analyses and rejections of imported food in Japan

Year	Notifications	Year ratio	Weight	No. of total analyses	Analysis sites (laboratories)			No. rejected
					Government	Private	Official foreign	
	(cases)	(%)	(1 000 tonnes)			(cases)		
1965	94 986	–	12 765	–	5 574	–	–	679
1975	246 507	–	20 775	–	21 461	–	–	1 634
1985	384 728	105.6	22 665	39 817	14 892	26 054	1 904	308
2000	1 550 925	110.5	30 034	112 281	52 244	63 789 (37 484)	3 796	1 037
2005	1 864 412	104.1	33 782	189 362	66 147	125 083 (73 589)	7 919	935
2006	1 859 281	99.7	34 096	198 936	61 811	139 991 (87 779)	6 953	1 530
2007	1 797 086	96.7	32 261	198 542	58 299	144 846 (94 598)	5 818	1 150
2008	1 759 123	97.9	31 551	193 917	58 706	140 878 (95 490)	6 208	1 150
2009	1 821 269	103.5	30 605	231 638	56 518	184 726 (110 308)	5 925	1 559
2010	2 001 020	109.9	31 802	247 047	57 359	195 954 (118 721)	6 200	1 376
2011	2 096 127	104.8	33 407	231 776	58 941	180 023 (99 117)	5 546	1 257

There are 31 quarantine offices in Japan where import notifications are submitted, examined and accepted. In 2009, 368 food inspectors with tertiary qualifications in agriculture, chemistry, veterinary science, livestock, fisheries or food science and technology were involved with document inspections, on-site inspections and chemical and microbiological examinations (MHLW, 2010b).

The test scheme for imported food is illustrated in Figure 61. If during monitoring tests, violations of the Food Sanitation Law, such as exceeding MRLs of pesticides or veterinary drug residues as in the example in the figure, are identified for the first time in a specific food item imported from a specific country, the food-pesticide / veterinary-drug / exporting-country combination will be the subject of strict monitoring. This means that 50 percent of the notified food / exporting-country combination will be analysed for the pesticide / veterinary drug in the specific food. If a second violation case (e.g. exceeding the MRL) is identified, the same food-pesticide / veterinary-drug / exporting-country combination will then be the subject of mandatory 100 percent testing, because it is considered that the same food-pesticide / veterinary-drug / exporting-country combination has a high probability of exceeding the MRL in the future.



In order to lift the mandatory test once mandatory inspections have been implemented, it is necessary to implement control measures in the exporting country to eliminate the risk factors associated with the series of violations, and for food safety authorities of the exporting country to provide evidence of effective implementation of control measures.

As shown in Table 87, in fiscal year 2011, 6 482 seafood and 15 943 processed seafood samples were taken for monitoring tests for veterinary drug residues, food additives and food standards including microbiological criteria, and only 38 violations were identified.

7.4.6 Results of imported-seafood inspections

The list of mandatory inspection food/country/hazard combinations associated with seafood is summarized in Table 88 (MHLW, 2011a). Forty-one seafood/hazard/country combinations were under mandatory inspection, which means 100 percent of the notifications of the food/country combination in this list must be examined for the hazards to show compliance with the Food Sanitation Law and related specifications and standards. Illegal and excessive levels of veterinary drugs detected in the food trigger the majority of mandatory tests.

TABLE 87
Annual monitoring plan, Japan (fiscal year 2011)

Food type	Analyses undertaken	Number of samples tested	Number of violations
Meat (beef, pork, chicken, etc.)	Veterinary drugs, pesticides, food standards, etc.	9 381	1
Processed food of animal origin (natural cheese, meat products, ice cream, etc.)	Veterinary drugs, food additives, food standards, etc.	7 806	9
Seafood (bivalves, fish and shellfish, crustaceans)	Veterinary drugs, food additives, food standards, etc.	6 482	7
Processed seafood (surimi, frozen fish, fish roe, etc.)	Veterinary drugs, food additives, food standards, etc.	15 943	34
Agricultural products (grain, vegetable, fruit)	Pesticides, food additives, aflatoxin, genetically modified organisms	20 866	44
Processed agricultural products	Pesticides, food additives, food standards, aflatoxin, genetically modified organisms, etc.	20 400	33
Other food (soup, seasoning, oil, snacks, confectionery)	Food additives, food standards, etc.	5 248	9
Drinks (mineral water, soft drinks, alcoholic drinks)	Food additives, food standards, etc.	2 473	1
Food additives, utensils, etc.	Food standards, etc.	2 731	18
50% monitoring foods		5 000	
Total		91 330	156

TABLE 88
Outcomes of seafood inspection from mandatory (100%) inspection, Japan – 40 food/country/hazard combinations

Hazard	Number
Veterinary drug residues	18
Food additives	2
Foreign matter	1
Pesticide residues	8
Paralytic shellfish poisoning	2
Paralytic shellfish poisoning and diarrhoeic shellfish poisoning	2
<i>V. parahaemolyticus</i>	4
<i>Salmonella</i> spp.	1
Shigella	1
Puffer fish	1
Nitrogen dioxide in ikura	1
Total (as of 14 April 2011)	41

In 2006, 195 violations were identified from imported fish, shellfish and processed seafood through monitoring tests and examinations at the port of entry (Table 89). When violations were found during regular inspections at quarantine stations and the foods were held in the designated bonded area, those foods were not permitted to be imported into Japan. Where the violations were identified during the monitoring test and foods had been imported into Japan, the foods were recalled and withdrawn from the market.

A total of 131 (67.2 percent) of the violations were due to veterinary drug residues, and 69 out of 131 (52.7 percent) veterinary drug violations were due to detection of chloramphenicol in squid and shrimp. Except for one case, all products were exported from Viet Nam. Out of 131 veterinary drug violations, 23 were due to detection of AOZ from shrimp shipments from India, Indonesia and Viet Nam, eel shipments from China and Taiwan Province of China and a crab shipment from China. Out of the 131 veterinary drug residues violations, 12 were due to the detection of leucomalachite green in eels and frozen baked eel imported from China.

TABLE 89

Violations of the Food Sanitation Law, Japan – cases involving seafood (2006 and 2007)

Hazard	Violations			
	2006		2007	
	Number	Specific hazard / number	Number	Specific hazard / number
Natural toxin	19	Diarrhoeic shellfish poisoning	8	Diarrhoeic shellfish poisoning
		Paralytic shellfish poisoning	2	Paralytic shellfish poisoning
		Puffer fish	6	Puffer fish
		Ciguatera	3	Ciguatera
Microbiological criteria	28	Bacterial count	7	Bacterial count
		Coliform group	18	Bacterial count & coliform group
		<i>Vibrio parahaemolyticus</i>	3	Coliform group
Veterinary drug residues	131	AOZ	23	AOZ
		AMOX	1	AMOX
		AOZ & semicarbazide	2	Endosulphan
		Endosulphan	10	Enrofloxacin
		Chloramphenicol	69	Over-the-counter drugs
		Semicarbazide	2	Chloramphenicol
		Tetracycline	5	Chloramphenicol & semicarbazide
		Malachite green	6	Semicarbazide
		Leucomalachite green	12	Chlortetracycline
		Leucomalachite green & AOZ	1	Tetracycline
				Malachite green & leucomalachite green
				Leucomalachite Green
Food additives	15	Tertiary butylhydroquinone	2	EDTA2Na
		Cyclamic acid	2	Sulphite
		Sulphite	6	Nitrite
		Nitrite	5	Carbon monoxide
				Hydrogen peroxide
				Sodium dehydroacetic acid:
				Boric acid (caviar) ¹
Pesticide residues	1		10	Endosulphan
				Chlorpyrifos
Others	1		1	
Total	195		209	

Note: AMOX = 3-amino-5-morpholinomethyl-1,3-oxazolidin; AOZ = 3-amino-2-oxazolidinone; EDTA2Na = ethylene diamine tetraacetic acid disodium.

¹ Boric acid is not allowed for use in caviar in Japan.

Regarding microbiological criteria, coliforms were detected in 13 cases of cooked surimi products imported from various Asian countries and in 5 lots of frozen, boiled octopus. In three cases, an MPN for *Vibrio parahaemolyticus* of more than 100 MPN/g was detected in two lots of ark shell from the Republic of Korea and one lot of tilapia from Thailand, both intended for raw consumption (MHLW, 2007).

In 2007, 209 violations were identified from fish, shellfish and processed seafood through monitoring tests and examinations at the port of entry (Table 89). Of the violations, 136 (65.4 percent) were due to veterinary drug residues, and 67 out of 136 (49.3 percent) veterinary drug violations were due to detections of chloramphenicol in squid and shrimp, and except for two cases all were exported from Viet Nam. Out of 136 veterinary drug violations, 34 were due to detection of AOZ in shrimp shipments from Indonesia and eel shipments from Taiwan Province of China. Of 136 veterinary drug residues violations, six were due to the detection of leucomalachite green in frozen, baked farmed eel, wild Chinese perch and frozen cut mackerel imported from China.

Regarding microbiological criteria, coliforms were detected in six cases of cooked surimi products imported from various Asian countries (in four cases of frozen, boiled octopus and in two cases from frozen fish for raw consumption). No cases of exceeding MPN levels (more than 100 MPN/g) of *Vibrio parahaemolyticus* were detected (MHLW, 2008).

More recently, in 2009, 233 violations were identified from imported fish, shellfish and processed seafood through monitoring tests and examinations at the port of entry. Of the violations, 64 (27.5 percent) were due to veterinary drug residues, and of these, 19 were due to detection of AOZ in shrimp, eels and crab shipments from various Asian countries. Of these 64 veterinary drug residues violations, four were due to the detection of leucomalachite green in frozen, baked farmed eel, wild Chinese perch and frozen cut mackerel imported from China.

Chloramphenicol was detected from one clam shipment imported from China and ten shrimp and eight squid shipments from Viet Nam. One hundred and twenty-eight violations were due to non-compliance with microbiological criteria (MHLW, 2010b).

7.4.7 HACCP

Traditionally, Japanese seafood processors only implemented GHPs, based on the Codex General Principles of Food Hygiene. However, since the late 1990s, the HACCP system has been introduced into the Japanese food industry. Especially at the start of this shift to use of the HACCP system, seafood processors that wanted to export their products to the European Union (Member Organization) and the United States of America were the trigger for the introduction of the HACCP system into Japan. All the seafood establishments exporting to the European Union (Member Organization) were certified by the Ministry of Health, Labour and Welfare, and a part of those for the United States market were also certified by the Ministry of Health, Labour and Welfare. Below is the number of the seafood establishments certified by the Ministry of Health, Labour and Welfare (MHLW, 2011b, 2011c).

- Certified seafood processors exporting to the European Union (Member Organization): 28 establishments.
- Certified seafood processors exporting to the United States of America: 74 establishments.

Furthermore, in 1995, the Ministry of Health, Labour and Welfare introduced, on a voluntary basis, a GHP/HACCP certification programme under the Food Sanitation Law (Article 13). At this time, among fish and fishery products, only surimi products and canned seafood were designated as target food categories for this certification programme. In April 2013, 23 surimi establishments were certified.

7.4.8 Positive list system

On 29 May 2006, the Ministry of Health, Labour and Welfare introduced a “positive list system” for agricultural chemical residues in foods in order to prohibit the distribution of foods that contain agricultural chemicals (including pesticides, feed additives and veterinary drugs) above 0.01 ppm, where MRLs have not been established.

Before this date, foods found to contain chemicals were not prohibited for sales if an MRL for the chemical had not been established.

Now, foods in which any agricultural chemical residues are found in excess of 0.01 ppm should not be produced, imported, processed, used, cooked, stored for sale, or sold. In addition, if the specific residue limit levels of chemical substances in certain food categories are articulated in the compositional specification for foods separately, the specific MRLs should be applied.

This amendment was based on the fact that if a pesticide was detected at a certain level from a processed food, but there was no established MRL for the processed food, there were no legal powers to recall or withdraw the food from the market. For this

reason, the uniform limit was introduced in the “positive list system”. Now, if more than 0.01 ppm of veterinary drugs and/or pesticides are detected in seafood, even without an MRL for the chemical, the seafood is prohibited from sale and distribution.

7.5 AUSTRALIA AND NEW ZEALAND (ALLAN BREMNER)

7.5.1 Introduction

7.5.1.2 Food standards

In recent years, the food regulatory and legal systems in Australia and New Zealand have been harmonized through the instrument of a Joint Food Standards Treaty to provide a joint food standards system covered by the Australia New Zealand Food Standards Code. As a joint authority, Food Standards Australia New Zealand (FSANZ) has been formed to draw up the science-based standards and to administer this code. Each country retains some independent functions within its own “Food Act” for differing local situations and the agencies involved may have different names and structures. The code, which covers all foods, domestic and imported, can be obtained online and on CD-ROM by subscription and in an unofficial consolidated form through FSANZ. Amendments to the code are published and obtainable for legal purposes through FSANZ.

7.5.1.3 Imports

The two nations are differently constituted in that Australia is a federation of separate states, each with its own food authority and legislation, but covered by a national set of import rules. New Zealand has no separate states and is a single sovereign entity under the one national authority.

Australia imports a considerable amount of seafood, mostly finfish, in processed, part-processed and RTE forms. New Zealand is more self-sufficient and is a net exporter of seafood, a large proportion of which goes to Australia.

Both nations are members of the WTO and adhere to its Arrangements, are signatories to the SPS Agreement, are members of the CAC and have free-trade agreements and mutual recognition arrangements with other countries.

The two countries have signed a Trans-Tasman Mutual Recognition Act, which allows food products made in or imported into one country, and which meet its legal requirements, to be also sold in the other. However, each country has a list of exemptions for products it considers to be high-risk. Because of their geographical isolation and historically recent settlement by Europeans, both nations are free from many of the animal and plant diseases that are endemic in the rest of the world. This status is protected by strict quarantine regulations so that the quarantine (biosecurity), customs and food authorities have to work very closely together.

Both countries have deliberately endeavoured to move away, as much as possible, from sampling, inspection and testing at the borders and to eliminate tests for potential contaminants that have proved to be insignificant, e.g. a review of years of analyses of prawns in New Zealand for heavy metal contamination revealed not one instance of imports being above permitted limits and, consequently, this is no longer a criterion. The two countries, in line with others across the globe, are adopting the more rational and effective approach of recognizing overseas controls on exported product where they meet, and are equivalent, to the required standards. This places the responsibility on the authorities that manage food safety and control in the exporting countries. In addition to placing emphasis on the foods that are classed as high-risk and ignoring those of lower risk, this risk-based approach seeks to classify all imported foods into the three groups of high-, medium- and low-risk and then establish standards to ensure their safety and reliability.

7.5.2 The situation in New Zealand

The New Zealand Food Safety Authority (NZFSA) is part of the Ministry of Food Safety, which has a wide brief and is part of the super Ministry for Primary Industries⁷³ (MPI) covered by the “Ministerial Statement of Responsibility: Statement of Intent 2013–2018”. This overarching MPI also incorporates the former Ministries of Agriculture and Forestry, thus including the quarantine system. The document *Imported Food and Food Related Products – A Blueprint for Change and Implementation* (NZFSA, 2007) is still current, having become effective in 2008. It has four major aspects:

- an Import Management Decision Making Framework, which covers the standards, risk management and the underpinning science;
- an Import System in which the mandatory requirements are described to ensure imported foods comply with the standards;
- a monitoring and review process to ensure the regime is kept up to date to meet changing circumstances;
- a communication programme to provide appropriate continuing communication to all parties involved.

The New Zealand Food Act sets out the standards for imported foods to which all foods must comply. The Minister for Food Safety is the responsible authority who can issue Prescribed Food Standards and Emergency Food Standards in addition to standards contained in the Joint Food Standard.

The role of coordination of public health units in the inspection, sampling and testing of high-risk foods is contracted to the Auckland Public Health Unit’s Central Clearing House.

The regime is based on the following premises that:

- The standard setting process will be based on scientific input.
- Mechanisms for standard setting will be reviewed, updated and developed in line with the New Zealand Standards Group.
- The Imported Food System covers all arrangements – delivery, determination of equivalences, pre-clearance arrangements with approved authorities in exporting countries and consultation.
- A consultative reference group is formed, comprising mainly government and importers.

Two major components are involved: (i) an Import Management Decision Making Framework, which includes risk profiling, risk assessment and ranking of all foods into high-, medium- and low-risk categories; and (ii) an Import System, which enables importers and all interested parties to comply with the standards efficiently and effectively.

A National Imported Food Programme and relevant Food Control Plans are designed to aid importers to meet the requirements of the standards, of which there will be four types: Generic Standards, Medium Interest Standards, High Interest Standards and Emergency Food Standards.

The Import System is based on the three principles that:

- the importer or import agency must be a New Zealand entity;
- the importer is responsible for ensuring the food is fit for purpose;
- the importer (or agent) must lodge correct and accurate documentation.

Importers and agents must be registered with the MPI, and any party that is unregistered must use a registered importer for “one-off” imports. Importers operate under a Food Control Plan, each of which is designed to be compatible with the New Zealand domestic Food Control Plan, thus reducing paperwork and providing access to the whole food chain. High-risk foods require pre-clearance arrangements before they can be imported. These can be where the country and/or exporter meet

⁷³ Ministry for Primary Industries (Manatū Ahu Matua) – www.mpi.govt.nz

the New Zealand standards or have equivalence, or where a pre-existing arrangement has been made. The competent export authorities must provide valid certification of their procedures. Verification, which may include sampling and testing, may occur at the border, coordinated through the New Zealand Customs Service and quarantine system.

Imports without clearance cannot be assured of entry if they are already in transit. Specific clearance permits may be applied for raw materials being imported for further processing and then exported. They will not be allowed to enter the domestic market. Returned exports must comply with New Zealand import standards.

Another aspect is the publication of a Scanning List detailing suspect foods or areas, complaints, incidents and listing. These will be given extra monitoring, and decisions made when and if they can be removed from the list.

In addition to meeting the requirements of customs and biosecurity, New Zealand importers have responsibilities under the Food Act 1981. Importers must ensure that products imported for human consumption are safe and suitable.

7.5.2.1 Prescribed foods

The NZFSA has specific options and clearance procedures available for importers of prescribed food. These procedures are known as imported food requirements. Persons who import food must ensure that the food complies in all respects with:

- all relevant provisions of the Food Act 1981;
- all relevant provisions of any regulations made pursuant to the Food Act 1981;
- all applicable food standards.

The NZFSA clearance options for prescribed foods may include:

- acceptance of recognized assurances/certification;
- clearance sampling and testing on arrival in New Zealand;
- multiple release permits.

The imported food requirements of prescribed foods to New Zealand are shown in Table 90.

TABLE 90
Imported food requirements for prescribed foods, New Zealand

Fish	Hazard
Fish – species susceptible to production of histamine	Histamine
Fish – manufactured fish products (surimi and marinara mix)	<i>Listeria monocytogenes</i>
Fish – smoked (vacuum packed)	<i>Listeria monocytogenes</i> and <i>Clostridium botulinum</i> Type E
Seafoods	Hazard
Bivalve molluscan shellfish	Metal contaminants, biotoxins, pathogenic bacteria and pathogenic viruses
Crustaceans – lobsters, crabs, bugs and their products	<i>Listeria monocytogenes</i> and <i>Salmonella</i> sp.
Crustaceans – shrimps and prawns	<i>Salmonella</i> sp., <i>Listeria monocytogenes</i> and other pathogens
Hijiki seaweed	Inorganic arsenic

Note: The commodities listed in the table can be found by following the links on the NZFSA webpage and searching for the commodity listed in the left-hand column of the table. Updates are available from: www.foodsafety.govt.nz/industry/importing/whats-new/

Source: www.foodsafety.govt.nz/industry/importing/guide/index.htm

7.5.2.2 Single Use Permits

Importers of prescribed food that is of interest to the NZFSA are referred to the Central Clearing House to apply for a “NZFSA Single Use Permit”. Application forms need to be emailed to the Central Clearing House. A Single Use Permit is the final

NZSFA clearance and permits full release to the domestic market. A Single Use Permit is issued if the Food Act Officer has been satisfied that the prescribed food complies with the Food Act.

7.5.2.3 *Conditional Release Permit*

If sampling and testing (or other evidence) is required, a Conditional Release Permit will be issued to allow the prescribed food to be moved to a holding facility. The condition of the release is that the prescribed food must be held until an officer is satisfied that the prescribed food complies with the Food Act. This may include provision of documentation, sampling and testing or inspection.

Control of product prior to final clearance, sampling and testing should be in accordance with the NZSFA's sampling and testing protocol (available from the NZSFA website⁷⁴).

7.5.2.4 *In practice*

In practice, customs are notified prior to importation and identify the product by its tariff code for inspection by an officer. The officer then examines the status of the origin, the supplier and the product itself and decides what further action is required.

The requirements for many products are similar and, as an example, those for surimi and marinara mix are shown in Box 4, which highlights the main components of the imported food requirements. The full details can be found on the MPI website for food safety.⁷⁵

7.5.2.5 *Summary*

New Zealand has a comprehensive and proactive system to deal with imports of seafoods and is transparent in these controls. All regulations, conditions, requirements, permit applications, details of tests and explanations are detailed and are available online.⁷⁶

7.5.3 **The situation in Australia**

The Australian system differs slightly in organization from that of New Zealand. All imported foods must meet the standards and requirements of the Australia New Zealand Food Standards Code, the same as for domestic foods. In addition they must also meet the requirements of state and territory legislation e.g. Fair Trading Acts.

The import area falls under the oversight of the Minister for Agriculture, Fisheries and Forestry and is administered through the Department of Agriculture, Fisheries and Forestry (DAFF) under the framework of the Imported Food Control Act 1992.⁷⁷ The Imported Food Control Regulations 1993 and Amendments made under the act govern the imports of all foods. The Imported Food Control Orders 2001 are issued under these regulations and contain various schedules and notices, as required for explanatory purposes or to meet changing circumstances, e.g. Imported Food Notice 05/11 dated 12 April 2011 – Testing of some Japanese food imports for radionuclides.

⁷⁴ www.foodsafety.govt.nz/industry/importing/

⁷⁵ www.foodsafety.govt.nz

⁷⁶ www.foodsafety.govt.nz/industry/importing/

⁷⁷ www.comlaw.gov.au

BOX 4

Example for imported food requirements for New Zealand**Fish: manufactured fish products (surimi and marinara mix)***Edited from detailed requirements***1.0 Scope**

Covering the purpose of the Imported Food Requirement, the legislative requirements, the clearance options and procedures for importers of prescribed foods, the specific products covered (in this case, surimi and marinara mix), the tariff codes targeted, the targeted food safety hazard and specific import conditions.

2.0 Importer clearance options

2.1 The clearance options and procedures in this Imported Food Requirement apply to manufactured fish products and are in addition to clearance requirements detailed in the Import Clearance Procedure.

2.2 Importers must also meet the requirements of the Food (Importer Listing) Standard 2008, the Food (Importer General Requirements) Standard 2008 and importers should also read and understand the Food Importer Standards Guidance before sourcing products to import.

2.3 For manufactured fish products, importers have a responsibility under the Food (Importer General Requirements) Standard 2008 to ensure that imports are not contaminated with pathogens. The processing method used in the manufacture of manufacture fish products can provide opportunities for *Listeria* contamination. Good manufacturing practices during processing can greatly reduce the likelihood of contamination. Manufactured fish products may not be heat treated prior to consumption, which would inactivate the pathogenic bacteria.

2.4 The following three options are available to importers:

Option 1 - Acceptance of recognised assurances / certification

Option 2 - Clearance sampling and testing on arrival in New Zealand

Option 3 - Multiple Release Permits (MRPs)

3.0 Clearance procedures

3.1 Certification checks

3.2 Physical inspections

3.3 Clearance sampling and testing on arrival in New Zealand

3.4 Multiple Release Permit (MRP)

3.5 Management of non-compliant consignments Consumer demand – reliable?

The regulations made under the act describe foods as being:

- risk food – food classified by FSANZ that has the potential to pose a high or medium risk to public health; or
- compliance agreement food – food to which a compliance agreement applies to the extent of the agreement; or
- surveillance food – food is classified as a surveillance food if it is not a risk food, not a compliance agreement food; or is the subject of a holding order.

These classifications are applied to imported food controlled through the Import Food Inspection Scheme, a joint service of FSANZ and the Australian Quarantine and Inspection Service (AQIS) of the DAFF, which is responsible for sampling and inspection control.⁷⁸

⁷⁸ www.daff.gov.au/biosecurity/import/food/inspection-scheme

The minister makes the orders that classify the foods into the groups; FSANZ supplies the advice behind these decisions and the authority makes recommendations to the minister.

Thus, FSANZ sets the standards and provides information on the category of risk for specific foods under the regulations, and AQIS ensures compliance with these standards through sampling and inspection. States and territory food enforcement agencies take physical responsibility for enforcing the requirements of the code for all food available for sale within their jurisdiction, including both imported and domestically produced food.

Upon entry of the food into the country, the Australian Customs Service inspects it according to the tariff code and determines whether it is in the risk, compliance or surveillance categories. Items classed as risk are all referred by the Australian Customs Service to AQIS for inspection. Those in the surveillance category may also be referred according to the nature of the items, or to their countries of origin, or to particular suppliers.

All consignments of risk foods are initially inspected against a range of hazards, but when five consecutive consignments have passed inspection, the inspection rate reduces to 25 percent, and after a further 20 consecutive passes the rate is further reduced to 5 percent of all consignments. However, it will return to 100 percent if the consignment fails an inspection, and it will remain at 100 percent until a history of compliance is re-established. Consignments that fail the 100 percent test are destroyed. Consignments of surveillance foods are inspected at a rate of about 5 percent on a test-and-release basis.

Imported food that does not comply with the standards may be subjected to a holding order, and the inspection rate rises to 100 percent to ensure that further imports meet requirements and that appropriate action has been taken. Generally, five subsequent consignments that comply are necessary to remove the holding order.

The amendment⁷⁹ to the Orders 2001 under Schedule 1 lists the following seafoods as risk foods:

- crustaceans, including prawns, that are cooked (whether or not chilled or frozen), but are not canned;
- fish of the following kinds:
 - tuna, including canned tuna (whether dried or not),
 - tuna products,
 - mackerel,
 - RTE finfish;
- marinara mix (whether or not chilled or frozen);
- bivalve molluscs (whether cooked or uncooked);
- seaweed – hijiki only.

The tests applied to risk foods are currently described in the DAFF website.⁸⁰

7.5.3.1 *Import conditions, permits and documentary requirements*

The conditions that imports must meet are contained in an Import Conditions database, named ICON, which is available online.⁸¹ This is an important website for all intending importers to obtain up-to-date information and from which to download applications to import foodstuffs.

Equally important, it is the website for the posting of alert notices from AQIS and for impending changes in permit conditions, e.g. Public Quarantine Alert PQA 0722 “Changes to uncooked peeled prawn import permit conditions”. In this alert, the

⁷⁹ Amendment FC2010C00170

⁸⁰ www.daff.gov.au/biosecurity/import/food/notices/2009/2012/ifn-0912

⁸¹ www.daff.gov.au/biosecurity/import/icon-icd

importer or authorized agent must inform AQIS of the white spot syndrome virus and yellow head virus test results by laboratory report before the release from quarantine of the consignment is considered.

The Minimum Documentary Requirements Policy and the Non-commodity Information Requirements Policy (relating to the packaging, container and cleanliness requirements, etc.) are also found in the AQIS database or through the link from ICON.

7.5.3.2 *Imported Food Consultative Committee*

The Imported Food Consultative Committee provides a consultative forum for a variety of stakeholders, including industry – a variety of different importers (ethnic foods, specialty foods, food for retail or further processing, seafood, fruits and vegetables), processors, analysts – and representation from AQIS, FSANZ and the DAFF Cargo Consultative Committee.

For finfish, the range of commodity categories and the conditions pertaining to their commercial import are listed in Table 91, which is an extract from ICON.

TABLE 91

A list of finfish commodities for human consumption in Australia and the countries to which the conditions apply

Commodity	Country
Finfish – consumer-ready form	All countries
Finfish – eviscerated, head off	All countries excluding New Zealand
Finfish – non-specified finfish	All countries excluding New Zealand
Finfish – specified and non-specified finfish	New Zealand
Finfish – specified finfish	All countries excluding New Zealand
Finfish – canned/retorted (family Salmonidae)	All countries
Fish – elasmobranch (shark, skates, rays)	All countries
Fish – uncanned/unretorted (family Salmonidae)	All countries excluding Canada, Denmark, New Zealand, Norway, Ireland, United Kingdom and United States of America

Source: Compiled from ICON, May 2011.

There is much common ground in many of the conditions. Despite the commonality, there are particular compliance requirements for each commodity.

These conditions often also apply to most non-commercial imports, but for some fish commodities small amounts of non-commercial imports are allowed without the need for an import licence, provided that:

- the fish is accompanied into Australia by the person importing it;
- the fish is imported in an amount up to 5 kg;
- the fish is eviscerated (gilled and gutted) or processed further than evisceration; and
- the product is for human consumption only.

For unaccompanied consignments, and consignments of greater than 5 kg, the commercial conditions must be met. These conditions are for human consumption only. If the fish is being used for aquaculture, bait or animal food, an import permit is required.

These conditions for commercial imports apply to the importation from all countries, other than New Zealand, of non-salmonid finfish that have had the head, gills and viscera removed and of further processed product that does not meet AQIS's specifications for consumer-ready product. Box 5 provides an example of the conditions required for consumer-ready finfish.

BOX 5

**Example of conditions that apply to finfish in consumer-ready form
– imports into Australia**

An example of the conditions (from ICON) that apply to finfish in the consumer ready form follows and that applies to all countries.

1. An Import Permit is not required for non-salmonid, consumer ready finfish products for human consumption. This includes finfish flesh including attached bone, cartilage, skin and blood. An Import Permit is required for fish products imported for aquaculture, animal feed or bait.
2. Consumer ready product is defined as product that is ready for the householder to cook/consume and includes the following examples:
 - (a) cutlets, including the central bone and external skin but excluding fins, each cutlet weighing no more than 450 grams; or
 - (b) skinless fillets, excluding the belly flap and all bone except the pin bones, of any weight; or
 - (c) skin-on fillets, excluding the belly flap and all bone except the pin bones, each fillet weighing no more than 450 grams; or
 - (d) eviscerated, headless 'pan-size' fish, each fish weighing no more than 450 grams; or
 - (e) fish that is headless and eviscerated which has been salted, dried or smoked, of any weight; or
 - (f) product that is processed further than the stage described in points 2 a) to e), including commercially canned product.
3. Each consignment must be packed in clean and new packaging and must be free of live insects, seeds, soil, mud, clay, animal material (such as faeces), plant material (such as straw, twigs, leaves, roots, bark) and other debris prior to arrival in Australia.
4. Consignments should be packaged to facilitate import inspection.
5. All consignments must be accompanied by documentation to verify that the product is in a consumer ready form as outlined above. Documentation may be in the form of an invoice, manufacturer's declaration or government health certificate. All documentation must be consignment specific. Scientific names are not required, however if the quarantine officer cannot determine from the common name that the fish is a non-salmonid species, documentation stating the scientific name may be requested.
6. Where consignments are not covered by valid documentation or are covered by documentation with an incorrect statement, consignments will be subject to inspection to ensure that the goods are in consumer ready form. An inspection fee will apply.
7. All consignments of dried or salted fish, must be inspected on arrival to ensure freedom from contamination and/or infestation by extraneous materials. If contamination and/or infestation is found, the material will be treated by an AQIS approved method, as applicable to the type of contamination (outlined in C9911 'Treatment of contaminants').
8. Where consignments do not fit any of the consumer ready categories above, refer to other ICON cases for finfish. Alternatively, the Biologicals Program, Canberra office may be contacted by email, phone (02 6272 4578) or fax (02 6249 1798) for further advice.
9. Timber packaging, pallets or dunnage in FCL containers will also be subject to inspection and treatment on arrival, unless certified as having been treated by an AQIS approved method (refer to the AQIS publication 'Cargo Containers - Quarantine aspects and procedures').

Finfish products from New Zealand are generally excluded from the requirements. For salmonid species, a range of countries (including Canada, Denmark, New Zealand, Norway, Ireland, the United Kingdom of Great Britain and Northern Ireland, and the United States of America) enjoy an exclusion from permit conditions. Other conditions that relate to packaging, processing and sale are included and must be met.

Molluscs and crustacea are often associated with greater hazards of contamination in that they often reside in the sediment and/or are filter feeders and, thus, safety is very dependent on environmental conditions. The requirements for mollusc and crustacean products for human consumption listed in ICON are presented in Table 92.

TABLE 92

A list of seafood commodities, other than finfish, including molluscs and crustacea for human consumption in Australia and the countries to which the requirements apply

Commodity	Country
Molluscs	
Marine molluscs other than oysters or snails (note: this category includes octopus and squid)	All countries
Oyster meat	All countries
Oysters in full shell – dead	All countries
Oysters in half shell – dead	All countries excluding New Zealand
Oysters in half shell – dead	New Zealand
Crustacea	
Crustaceans (other than brine shrimp eggs, raw prawns, raw freshwater crayfish and crustacean meal)	All countries
Foods – re-imported Australian foods (excluding whole seeds and fresh fruit and vegetables)	Australia
Prawns – cooked or dried	All countries
Prawns – uncooked, whole, partially peeled, peeled or highly processed	New Caledonia
Uncooked prawns–highly processed	All countries excluding New Caledonia
Uncooked prawns – peeled	All countries excluding New Caledonia
Uncooked prawns – whole or partially peeled	All countries excluding New Caledonia
Scampi	All countries

Source: Compiled from ICON May 2011.

Requirements and exclusions for commodities that are not for human consumption, such as for use in pet food, fertilizer, bait, aquaculture, human therapeutics, complementary medicine or cosmetics, are searched for separately in ICON and are not listed here.

7.5.4 Conclusion

Australia and New Zealand have strict quarantine, biosecurity and safety provisions to safeguard their disease-free status and protect their consumers.

As a closing statement, this section provides an overview of the major requirements for importing seafoods into Australia and New Zealand, including some examples, but much more detail is available from the webpages of the relevant authorities. These details include such matters as the egg and dairy content of batter composition, the protocol for fumigation of wooden pallets, the cleanliness of shipping containers, lists of test protocols, restricted species related to resource protection (e.g. Patagonian toothfish), and so on.

The safety of the imported food supply and the prevention of the spread of non-indigenous diseases are of considerable concern to the governments of Australia and New Zealand. Therefore, both countries have comprehensive, science-based requirements relating to the import of seafoods.

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APPENDIX 1

Example of a hazard analysis worksheet

Firm Name: Firm Address:			Product Description: Method of Storage and Distribution Intended Use and Consumer		
(1)	(2)	(3)	(4)	(5)	(6)
Ingredient/ processing step	Identify potential hazards introduced, controlled or enhanced at this step	Are any potential food-safety hazards significant? (yes/No)	Justify your decisions for column 3.	What preventive measures can be applied to prevent the significant hazards?	Is this step a critical control point? (yes/No)
Step 1	Biological Chemical Physical				
Step 2	Biological Chemical Physical				
Step 3 (etc.)	Biological Chemical Physical				

APPENDIX 2

Summary of HACCP plan development for canned fish

The HACCP plan of company XYZ is presented in Table A2.1. Where necessary, the table refers to annexes that describe in sufficient detail the relevant control, monitoring or corrective action or to record-keeping forms. The annexes to this appendix are as follows:

- Annex A2.I: Control measures adopted by company XYZ
- Annex A2.II: Monitoring system of the company XYZ – some examples.
- Annex A2.III: Record-keeping at the company XYZ – an example form.

The following is the hazard analysis and ensuing steps of CCP identification and development of critical limits, monitoring procedures and corrective actions that was applied to the production flow diagram (Figure 45 in the main text of the document to which this is an appendix) and resulted in the summary HACCP plan (Table A2.1).

Step 1: Receiving of fish at the plant

HAZARD 1: Fish with high histamine content.

Control measures:

1. Purchase of fish with acceptable freshness.
2. Proper fish icing.
3. Refrigerated truck transportation.
4. Regular maintenance of the truck's refrigeration system.
5. Histamine analysis at receiving and reject lots with unacceptable levels of histamine.

Is step 1 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes. See measures 1–5 above.
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	No.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	Yes. A delay during unloading can lead to further histamine accumulation
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	No. Once in fish, histamine cannot be removed.
Conclusion	Receiving fish is a CCP where the hazard of high histamine levels can be controlled.

Critical limit:

1. Temperature < 5 °C.
2. Fish freshness grade > 1.5.
3. TVB < 25 mg-N/100 g.
4. Histamine content < 7 mg/100 g.

Monitoring procedure:

1. Fish temperature is checked.
2. Fish freshness is assessed.
3. TVB is determined.
4. Histamine is analysed.

Corrective action:

1. If the lot has an average freshness score higher than the critical limit but acceptable TVB and histamine levels, qualified workers will sort fish and keep only the good-quality ones, under the supervision of the QC manager.
2. If the lot has high TVB or histamine levels, it will be rejected, unless it is proved chemically that some sublots are acceptable in terms of TVB and histamine levels.

Verification procedure:

See section 6.4.13.12.

Record-keeping:

See section 6.4.13.13 (also see Annex A3.III Form 13 in Appendix 3).

Step 2: Receiving empty cans

HAZARD 1: Contamination of finished products, with pathogens or toxic materials, because of leaking or dirty cans.

Control measures:

1. Verification of cans upon reception and before utilization.

Is step 2 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes, verification of cans upon reception and before utilization.
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes. The systematic verification of each lot of empty cans will reduce the likely occurrence of the hazard.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	Checking cans is critical to control post-process contamination.

Critical limit:

1. Only acceptable cans will be cleaned and stored.

Monitoring procedure:

1. Visual verification of 5 containers per pallet received from supplier and of 5 empty containers per 30 min, during packing.

Corrective action:

1. Refuse pallets with defective containers.
2. Isolate and inspect containers closed since last control and discard defective ones.

Verification procedure:

See section 6.4.13.12.

Record-keeping:

See section 6.4.13.13 (also see Annex A3.III Form 13 in Appendix 3).

Step 3: Preparatory steps (heading, gutting, brining)

HAZARD 1: Contamination of fish by, and multiplication of, pathogenic bacteria.

Control measures:

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex A2.I). Some relevant SSOP monitoring records are presented in Annex A2.III.

Step 4: Mechanical marking of can lids

HAZARD 1: Post-process contamination because of leakage following micro-puncturing of the lid.

Control measures:

1. Maintenance of the mechanical marking equipment.

Is step 4 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes. Regular maintenance of the mechanical equipment used for marking lids.
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes. Regular maintenance of the mechanical equipment will allow proper marking of lids.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	Lid marking is a CCP where proper maintenance of the equipment will allow proper marking of the lids.

Critical limit:

1. Acceptable non-leaking markings.

Monitoring procedure:

1. Check visually marking of 10 lids at the beginning of each production.

Corrective actions:

1. Find the reason for bad marking and remedy to the situation.
2. Isolate all suspect cans and verify one by one. Discard any can likely to be leaking.

Verification procedure:

See section 6.4.13.12.

Record-keeping:

See section 6.4.13.13 (also see Annex A3.III Form 13 in Appendix 3).

Step 5: Packing fish manually in cans

HAZARD 1: Contamination of fish by, and multiplication of pathogenic bacteria.

Control measures:

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex A2.I). Some relevant SSOP monitoring records are presented in Annex A2.III.

Step 6: Rinsing packed fish

HAZARD 1: Contamination with pathogenic bacteria from water.

Control measures:

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex A2.I). Some relevant SSOP monitoring records are presented in Annex A2.III.

Step 7: Loading cans on trays and carts

There is no significant chemical, physical or biological safety hazard at this step.

Step 8: Cooking

There is no significant chemical, physical or biological safety hazard at this step.

Step 9: Draining of cooked fish

HAZARD 1: Cross-contamination of fish and multiplication of pathogenic bacteria, especially *S. aureus* as cooking has destroyed the normal fish flora.

Control measures:

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex 2.I). Some relevant SSOP monitoring records are presented in Annex 2.III.

Step 10: Filleting mackerel-based products or removing skin and bones for skinless boneless sardines

HAZARD 1: Contamination by workers of fish and multiplication of pathogenic bacteria, especially *S. aureus* as cooking has destroyed the normal fish flora.

Control measures:

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex 2.I). Some relevant SSOP monitoring records are presented in Annex 2.III.

Step 11: Hot filling

There is no significant chemical, physical or biological hazard at this step.

Step 12: Container closure

HAZARD 1: Leaking containers, leading to contamination and growth of pathogenic bacteria.

Control measures:

1. Training of container closure equipment supervisor.
2. Regular maintenance of the container double-seaming equipment.

Is step 12 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes. Training of container closure equipment supervisor. Regular maintenance of the container closure equipment.
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes, Proper container closure will eliminate the risk of re-contamination of packed products.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	Container closure is a CCP where proper seaming operating procedures will eliminate the risk of re-contamination of finished product.

Critical limits:

1. Certified and experienced container closure equipment supervisor.
2. Well maintained container closure equipment.
3. Non-leaking containers (see Annex A2.I for double-seaming standards).

Monitoring procedure:

1. At the start of each shift, check 5 closed containers.
2. Afterwards, inspection of 5 cans every 30 min and a detailed verification of a can every two hours.

Corrective actions:

1. If a closure defect is detected, the operator will stop the machine, check the cause and make the appropriate adjustments and controls.
2. The supervisor will inspect all containers closed since the last inspection and discard any suspect ones.

Verification procedure:

See section 6.4.13.12.

Record-keeping:

See section 6.4.13.13 (also see Annex A3.III Form 13 in Appendix 3).

Step 13: Washing seamed cans**HAZARD 1: Contamination with pathogenic bacteria from water.****Control measures:**

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex A2.I). Some relevant SSOP monitoring records are presented in Annex A2.III.

Step 14: Sterilization**HAZARD 1: Survival of pathogenic bacteria, especially *C. botulinum* spores, which may germinate later on, grow and produce their deadly neurotoxins.****Control measures:**

1. Regular maintenance of the sterilization equipment.
2. Training of the retort operating supervisor.

Is step 14 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes. Regular maintenance of the sterilization equipment. Training of the retort operating supervisor.
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes, proper sterilization will inactivate all bacteria, including <i>Cl. botulinum</i> spores.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	Sterilization is a CCP for the elimination of bacterial or spore survival.

Critical limits:

1. $F_0 = 7\text{--}14$ min, certified and registered thermal process.
2. Well-maintained retorts.
3. Certified retort operation supervisor through, for example, BPCS or equivalent.

Monitoring procedure:

1. Run twice a year, and as seen fit, a heat penetration and distribution test on each retort.
2. For each retort cycle, record automatically time–temperature data on a thermograph, and record manually temperature at the mercury thermometer, pressure at the manometer, time for steam-on, steam-off, beginning and end of sterilization, expected sterilization time and temperature.

Corrective actions:

1. Identify the cause of underprocessing and solve the problem.
2. Re-sterilize if acceptable or discard and destroy understerilized product.

Verification procedure:

See section 6.4.13.12.

Record-keeping:

See section 6.4.13.13 (also see Annex A3.III Form 13 in Appendix 3).

Step 15: Cooling hot sterile cans (only when vertical discontinuous retorts are used. Not applicable for Steriflow)

HAZARD 1: Post contamination of can content with pathogenic bacteria from water.

Control measures:

1. This hazard is reduced to acceptable levels through strict adherence to the company's SSOP (Annex A2.I). Some relevant SSOP monitoring records are presented in Annex A2.III.

Step 16: Storage before shipment

There is no significant biological, chemical or physical safety hazard at this step.

TABLE A2.1
HACCP plan for the production of canned fish

Critical control point(s)	Significant hazard(s)	Preventive measure(s) (Annex A2.I)	Monitoring procedure(s) (Annex A2.II)				Corrective action(s)	Records (Annex A2.III)	Verification
			What	How	Frequency	Who			
Receiving fish at the plant	Spoiled fish	Proper fish icing and handling	Fish temperature < 5 °C	Thermometer	10 boxes/lot	Reception and QC supervisors	Fish sorting according to freshness. If needed, determination of TVB	Fish receiving log	Daily record review
			Freshness > 1.5	Scorecard	Sampling plan				
			TVB < 25 mg-N/100 g	TVB test	10 fishes/lot				
Receiving empty cans	Fish with high histamine levels	Proper fish icing, refrigeration and handling	Fish temperature < 5 °C	Thermometer	10 boxes/lot	Reception and QC supervisors	Fish sorting according to freshness. Reject fish lots with high histamine levels	Fish receiving log	Daily record review
			Histamine < 70 ppm	Histamine test	10 fishes/lot				
Mechanical marking of can lids	Post-process contamination because of leakage	Inspection of containers (per lot) at receiving	Clean and non-leaking containers	Visual	5 containers per pallet	Container closure inspector	Refusal of any pallet with damaged cans	Container inspection log	Review records at every reception
			Clean and non-leaking containers	Visual	5 containers every hour	Container closure inspector	Isolate the lot of containers used since the last verification and discard any leaking ones	Container inspection log	Daily record review
			Acceptable and non-leaking marking	Visual verification	10 lids at the start of marking	Container closure inspector	Isolate cans used since last verification and discard any leaking ones	Lid marking inspection log	Daily record review
Container closure	Post-process contamination	Training of the container closure supervisor.	Visible defects of cans	Visual inspection	At the start of a shift (5 cans) and every 30 minutes thereafter	Container closure inspector	Isolate cans closed since last inspection and discard any leaking ones	Container integrity records	Daily record review
			No leaking containers	Double seam analysis	Every 2 hours	Container closure inspector	Isolate cans closed since last analysis and discard any leaking ones. Check and adjust equipment	Container integrity records	Daily record review
			Heat penetration and distribution	Standard heat penetration and distribution test	Twice a year and as seen necessary	QC manager	Identify cause of deficiency and repair the retort	Automatic recordings	Review at every run
Sterilization	Survival of spores, especially those of <i>Cl. botulinum</i>	Regular maintenance of the retorts	Fo = 7–14 min	Heat penetration and distribution	Every retort	Retort operator	Re-sterilise or destroy.	Sterilization log	Daily record review
			Temperature, time, pressure	Thermograph recordings					
			Sterilization parameters						

ANNEXES

As indicated above, the HACCP plan contains three annexes developed by the HACCP team to address, respectively: control measures, monitoring procedures, and record keeping. The table of contents of each annex is presented below, along with some elements of each annex for illustrative purposes only.

ANNEX A2.I: CONTROL MEASURES

Company XYZ has adopted control measures to promote the application of good hygiene, handling and sanitation practices by the employees and good manufacturing practices during processing.

The control measures used at company XYZ comprise:

- standard sanitation operating procedures (SSOPs);
- standard handling and icing of fresh fish;
- standard sterilization procedure;
- maintenance of container closure equipment;
- maintenance of sterilization retorts.

Examples to illustrate the content of Annex A2.I are provided here for illustrative purposes only.

Standard handling and icing fresh fish

Before buying fish, the purchase supervisor checks the freshness of fish. The quality control (QC) manager and the purchase supervisor are in close contact to ensure that only quality raw material is purchased. Before loading the truck, fish is iced in 25 kg plastic boxes, by alternating a layer of ice and a layer of fish.

Ice is purchased from reliable suppliers that use potable water and the appropriate containers. The ratio of fish to ice depends on whether the truck is refrigerated or insulated and on the transport duration. These ratios are as shown in Table A2.2.

TABLE A2.2
The ratio of fish to ice in transport vehicles

Type of truck	Kilograms of ice to preserve 100 kg of fish for:				
	3 hours	6 hours	12 hours	18 hours	24 hours
Insulated	22 ⁽¹⁾ to 35 ⁽²⁾	38 to 45	40 to 65	53 to 85	65 to 105
Refrigerated	17 ⁽¹⁾ to 25 ⁽²⁾ kg of ice for 100 kg of fish regardless of the duration				

⁽¹⁾ This amount of ice is used when the outside temperature is relatively low, about 15 °C.

⁽²⁾ This amount of ice is used when the outside temperature is high, about 30 °C.

Fish temperature is kept around that of melting ice during transportation. The melted ice is allowed to drain freely. Hired trucks are generally not used for carrying other products that may contaminate fish. In the unlikely event of this happening, drivers are instructed to use these trucks to carry fish only after their thorough cleaning and sanitation

Standard sterilization procedure

Product sterilization at company XYZ uses three retorts of the type Steriflow. These retorts use cascading high-pressure water and recycle it, after a cooling step through the Steriflow heat exchanger, to be used to cool the cans. This eliminates the problem of post-process contamination from water and allows substantial savings in water consumption. On very rare occasions, sterilization is done using vertical retorts that operate under pressure in steam. Table A2.3 presents the technical parameters of thermal processing at company XYZ.

TABLE A2.3

Technical parameters of thermal processing

Technical parameters of the Steriflow retorts	Thermal processing target values
Retort type	Steriflow using overheated water
Minimal F_0	7 to 14 min, generally > 10 min
Can format	¼ P22 (115 g), 1/6 P 25 (125 g); 1/6 P 30 (125 g); ½ P oval (375 g); ½ H 40 (365 g); ½ HL (425 g); ½ B (425 g)
Sterilization temperature	122.5 °C, with an overshooting at 123.5 °C
Heating duration	23 to 55 min at 122.5 °C
Minimal initial temperature	30 °C
Filling method	Manual fish packing and overfilling with liquid
Ratio solid/liquid	75% fish; 25% liquid (oil, water, tomato sauce)
Stacking of the cans in the retort basket	In bulk
Number of basket per retort	4 baskets
Sterilization system	Water is overheated to a pressure of 6 bars
Cooling method	The heating water is cooled through a heat exchanger and recycled to be used for cooling
Technical parameters of the vertical steam retorts	Thermal processing target values
Retort type	Vertical retorts using steam
Minimal F_0	7 to 14 min, generally > 10 min
Can format	1/6 P 30 DAS (125 g); ½ HL (425 g)
Sterilization temperature	115 °C
Heating duration	55 to 85 min at 115 °C
Minimal initial temperature	30 °C
Filling method	Manual fish packing and overfilling with liquid
Ratio solid/liquid	75% fish; 25% liquid (oil, water, tomato sauce)
Stacking of the cans in the retort basket	In bulk
Number of baskets per retort	1 basket
Sterilization system	Introduction of steam through the by-pass. Venting for at least 10 min at 105 °C. Close the by-pass. open the regulating valve at temperature reaches 115 °C. This is start time.
Cooling method	Using cold water chlorinated at 2 ppm of active chlorine

The retorts are maintained at least once a year, under contract with the manufacturing company. Before resuming production and whenever needed, a heat penetration and distribution test is carried out to ensure proper functioning of each retort. The F_0 is calculated after each sterilization cycle, and records are kept for the duration of product shelf-life.

ANNEX A2.II: MONITORING PROCEDURES

The monitoring system of company XYZ comprises:

- control of cleaning and sanitation;
- determination of water chlorine level;
- measurement of fish temperature;
- sensory evaluation of fresh fish;
- determination of total volatile bases (TVB);
- determination of histamine;
- verification of the container closure;
- determination of the heat penetration and distribution.

Examples to illustrate content of Annex A2.II are provided hereafter for illustrative purposes only.

Determination of residual chlorine in water and in sanitizing solutions

Residual chlorine is measured using the Lovibond technique, whereby residual chlorine reacts with N,N-diethyl-p-phenylene-diamine (DPD) to form a stable red colour. The moulded flask containing 10 ml of the water sample is placed in the left compartment of the Lovibond apparatus. The other flask is first rinsed with the sample, before placing in few drops of the water to analyse. A tablet of DPD is added and left to react for a while, before completing to 10 ml with the water sample and placing it in the right compartment. The Lovibond apparatus is maintained in a vertical position, making sure the disc in the centre is facing the operator. The Lovibond apparatus is directed to a natural or artificial source of light and the disc is turned until the colour matches that of the sample in the right compartment, corresponding to the reading of concentration of residual chlorine.

Water disinfected in the plant should contain 1–2 ppm of residual chlorine. Municipal water is disinfected using 0.3–0.5 ppm of residual chlorine. The recommended chlorine levels of sanitizing solutions are described in the SSOP of company XYZ (Annex A2.I).

Measurement of fish temperature

Fish temperature is assessed on fish samples taken from about ten boxes chosen randomly from different areas of the delivery trucks (sides, centre, bottom, upper area). The warm fish is often in the centre of refrigerated trucks.

Only metallic thermometers are used. The probe is inserted as deep as possible in the fish through the anus. The reading is made as fast as possible once the temperature stabilizes. By so doing, one avoids errors due to heat conduction.

The warm areas in the truck are located, and the freshness of the fish stored in these areas is carefully assessed.

Thermometers are calibrated every three months in melting ice (0 °C) and boiling water (100 °C). Defective thermometers are automatically discarded.

ANNEX A2.III: RECORD-KEEPING

The HACCP plan (Table A2.1) developed by company XYZ refers to forms and logs to record respectively the results of monitoring and corrective actions.

The following is an example used to monitor quality of fish at receiving.

Example form: Control of fresh fish quality at company XYZ

Fish port: _____ Boat: _____ Transport truck: _____	
Date of purchase:	Date of receiving:
Quantity (tonnes):	Utilization:
<u>Temperature</u> Number of measurements: Range: Average temperature (°C):	<u>Fish freshness score (from 0 to 3)</u> Sample size: Range: Average:
TVB (mg-N/100 g):	Histamine (mg/100 g):
Storage conditions before utilization	Storage duration before utilization
Purchase manager	Quality control manager

APPENDIX 3

Summary of HACCP plan development for shrimp farm

The HACCP plan of company VWX is presented in Table A3.1. Wherever necessary, the table refers to annexes that describe in sufficient detail the relevant control, monitoring or corrective action or to record-keeping forms. The annexes to this appendix are:

- Annex A3.I: Good aquaculture practices at shrimp farm VWX.
- Annex A3.II: Monitoring system of the shrimp farm VWX – some examples.
- Annex A3.III: Record-keeping of the shrimp farm VWX – some example forms.

The following is the hazard analysis and ensuing steps of CCP identification and development of critical limits, monitoring procedures and corrective actions that was applied to the production flow diagram (Figure 57) and resulted in the summary HACCP plan (Table A3.1).

Step 1: Shrimp farm and surroundings

HAZARD 1: Presence of pesticides in shrimp as a result of the contamination from nearby agricultural farms.

Control measure(s):

The following GAP should be implemented to control this hazard:

1. Assessment of data available, including historical occurrence, types of pesticides, presence in soils and waters.
2. Soil and water testing.
3. No production when risk of contamination is high.
4. Eliminate the cause of contamination.

Is step 1 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes (measures 1– 4 described above)
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes. By applying the control measures described above
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	This step is a CCP for the production of shrimp free of pesticides

Critical limits:

1. Assessment of data indicative of no contamination risk.
2. Absence of pesticides in soil and water.
3. Cause of contamination eliminated/minimized before production resumes.

Monitoring procedure:

What	Expert evaluation of the results of the study. Laboratory analysis or rapid testing. Verification that the cause of contamination is eliminated/minimized.
How	Survey pollution sources: ask questions about and observe agricultural and industrial practices around the shrimp farm area. Investigate what pesticides are used on local harvested crops, how they are applied, and at what time of the year. Investigate what industrial discharges enter the watershed surrounding the shrimp farm area; prepare summary report of observations and findings. If water analysis is considered necessary, the method of analysis should be referenced and described in details as annex to the HACCP manual. Verify that soil and water analyses are acceptable.
Who	Person indicated by the farm management. Government official laboratory or certified private laboratory. QC manager.
When	Every three months or during agricultural treatment periods. Every three months or during agricultural treatment periods. Every three months or during agricultural treatment periods.

Corrective actions:

1. No shrimp farming in contaminated sites.
2. No shrimp farming if soil or water is contaminated.
3. No shrimp farming until source of contamination eliminated / contamination minimized.

Verification procedure:

See section 6.10.4.12.

Record-keeping:

See section 6.10.4.13 (also see Annex A3.III Form 13 in this appendix).

Step 2: Hatchery and grow-out

HAZARD 1: Presence of pathogenic bacteria (*Salmonella*, *Vibrio*, other) in shrimp because of contamination from workers, surroundings, domestic or wild animals and birds

Control measures:

The following GAP and hygienic practices are applied to control the hazard of biological contamination of shrimp during grow-out (production):

1. Hygienic practices should be strictly followed.
2. Training of workers on basic hygiene and health education.
3. Construction and maintenance of physical barriers to protect the ponds from livestock faecal contamination via water drainage.
4. Construction and maintenance of fences to protect shrimp ponds from domestic animals defecating in the ponds.
5. Establishment of a list of approved feed suppliers that provide assurance that feed is free of microbiological contamination.
6. Storage of shrimp feed in such a way and in a local where it is protected from microbiological contamination.

Is step 2 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes (measures 1–6 described above)
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	No. If contamination occurs, it will remain in the product throughout distribution
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	Yes, biological contaminants can subsequently grow to unacceptable levels
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	No. There is no designed step to remove bacterial contamination before shipment
Conclusion	This step is a CCP for the production of shrimp free of biological hazards

Critical limits:

1. No unhygienic practices during shrimp production.
2. All workers demonstrate a satisfactory understanding and application of hygiene rules.
3. Physical barriers and their maintenance in place.
4. Fences and maintenance in place.
5. Certified feeds from each supplier on the approved list.
6. Proper storage local and conditions.

Monitoring procedure:

What	Sanitation procedures. Personnel hygienic practices. Physical barriers and their state. Fences and their states. List of approved suppliers and feed certifications. Storage local and conditions.
How	Verification and supervision. Verification and supervision. Visual verification. Visual verification. Verification. Verification.
Who	Pond supervisors and QC manager. Pond supervisors and QC manager. Pond supervisors. Pond supervisors. QC manager. Pond supervisors.
When	Every week. When first hired and daily thereafter during production. Every production cycle. Every production cycle. When receiving feeds and before use. Weekly.

Corrective actions:

1. Re-train workers to improve hygienic practices and assess risk of contamination during the period of unsanitary practice.
2. Re-train workers, otherwise restrict duties to those that do not bring staff in contact with shrimp and production.
3. Repair barriers and assess risk of contamination when barrier was not operational.
4. Repair fence and assess risk of contamination when fence was not operational.
5. Not to use uncertified feed or feed from non-authorized supplier and assess cause and risk due to the use of uncertified feed or non-authorized supplier.
6. Improve storage conditions and assess risk when storage conditions were inadequate.

Verification procedure:

See section 6.10.4.12.

Record-keeping:

See section 6.10.4.13 (also see Annex A3.III Form 13 in this appendix).

HAZARD 2: Presence of unauthorized veterinary drugs or high residue levels of veterinary drugs in shrimp.

Control measures:

The following GAP is applied to control the hazard of unacceptable or high residue levels of veterinary drugs in shrimp hatchery and grow-out (production).

1. Only approved veterinary drugs should be used.
2. Veterinary drugs should be used under the supervision of a licensed professional.
3. Withdrawal times should be respected.

Is step 2 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes (measures 1–3 described above)
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes. If the above control measures are applied.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	This step is a CCP for the production of shrimp free of veterinary drug residues

Critical limits:

1. No unapproved veterinary drug is used (Annex A3.I) (WHO, 2006b).
2. All necessary veterinary treatments are supervised by a licensed professional.
3. Withdrawal times > those prescribed (e.g. 30 days for oxytetracycline).

Monitoring procedure:

What	Type of veterinary drug. Supervision of drug application. Withdrawal time and, if in doubt, check residues.
How	Verification. Verification of credentials of the treatment supervisor. Verification and, if in doubt, chemical analysis.
Who	Hatchery manager / pond supervisor. Pond supervisor. Pond supervisor and, if in doubt, analysis by certified laboratory.
When	During each treatment. During the treatment. After each treatment.

Corrective actions:

1. Investigate the cause of the use of unauthorized drug and ensure it does not happen again. Identify concerned tank/pond, observe withdrawal time or keep the product until a full food safety evaluation can be completed. If unfit for human consumption, divert product for non-food use or destroy it.
2. Investigate the cause of deviation and modify drug-use practice or change practitioner. Place suspect tank(s)/pond(s) on hold, observe withdrawal time and undertake full safety evaluation. If unfit for human consumption, divert product for non-food use or destroy.
3. Ensure that withdrawal duration is respected until analysis of veterinary drug residues is acceptable. Otherwise, keep the product until a full food safety evaluation can be completed. If unfit for human consumption, divert product for non-food use or destroy it.

Verification procedure:

See section 6.10.4.12.

Record-keeping:

See section 6.10.4.13 (also see Annex A3.III Form 13 in this appendix).

Step 3: Harvesting and transport

HAZARD 1: Presence of biological hazards (*Salmonella*, *Vibrios*, others) in shrimp because of bacterial contamination and growth during harvesting and transportation

Control measures:

The following GAP and GHP are applied to control the hazard of biological contamination of shrimp during harvesting and transportation.

1. Follow hygienic practices during harvesting and transportation.
2. Training of personnel on basic hygiene, health education and proper fish handling.
3. Use potable water for cleaning shrimp and preparing ice.
4. Use proper handling practices (harvest when temperature is cooled and under cover, avoid unnecessary delays, chill in sufficient ice or ice slurry immediately after harvesting, avoid physical damage, clean and disinfect surfaces that will come in contact with shrimp).

Is step 3 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes (measures described above)
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	No. If contamination occurs, it will remain in the product throughout distribution and growth may occur in subsequent steps
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	Yes, biological contaminants can subsequently grow to unacceptable levels
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	No. There is no designed step to remove bacterial contamination before shipment
Conclusion	This step is a CCP for the production of shrimp free of biological hazards

Critical limits:

1. All personnel demonstrate understanding and application of good hygiene and shrimp handling practices.
2. Only potable water is used for cleaning and production of ice.
3. Ice (or ice slurry)/ shrimp ratio so that fish reaches $\leq +5^{\circ}\text{C}$ within 3 hours (See Table A3.2, Annex A3.I).

Monitoring procedure:

What	Personnel hygiene and shrimp handling practices. Water quality. Ratio of ice/fish used or shrimp temperature.
How	Training, verification and supervision. Rapid test. Verification of weight of ice and shrimp or measuring temperature with a thermometer.
Who	QC manager. QC manager. Pond supervisors.
When	When first hired and as seen fit. Monthly (frequency to decrease or increase depending on the findings). Every shrimp box.

Corrective actions:

1. Re-train farmers/staff, otherwise restrict duties to those where untrained workers do not come in contact with shrimp harvesting.
2. Change water source or treat the water to make it potable. Keep on hold suspect lots and investigate food safety implications. If unfit for human consumption, divert product for non-food use or destroy it.
3. Add ice or ice slurry.

Verification procedure:

See section 6.10.4.12.

Record-keeping:

See section 6.10.4.13 (also see Annex A3.III Form 13 in this appendix).

HAZARD 2: Presence of unacceptable residue levels of sulphur dioxide (SO₂) as a result of improper treatment with metabisulphite

Control measures:

This hazard is controlled as follows:

1. Train farmers/staff on proper metabisulphite treatment, especially respect of the concentration of metabisulphite bath and treatment duration.
2. Use proper concentration of metabisulphite and proper duration.

Is step 3 a CCP for the considered hazard or not?

Question 1: Does any control measure exist for the identified hazard?	Yes (measures 1 and 2 described above)
Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level?	Yes. If the above control measures are applied.
Question 3: Could contamination with the identified hazard occur in excess of acceptable levels or could these increase to unacceptable levels?	–
Question 4: Will a subsequent step eliminate the identified hazard or reduce its likely occurrence to an acceptable level?	–
Conclusion	This step is a CCP for the production of shrimp with acceptable SO ₂ residue levels

Critical limits:

1. Only trained people will be in charge of preparing sodium metabisulphite baths and shrimp treatment.
2. Concentration 1.25 percent, duration 1–3 min.
3. SO₂ level < 100 ppm in raw shrimp.

Monitoring procedure:

What	Personnel practices and knowledge. Weight of metabisulphite / volume water and duration. SO ₂ concentration in bath and/or shrimp.
How	Verification and supervision. Verification of weight/volume water and duration. Rapid test to assess concentration in bath and/or shrimp
Who	QC manager. Pond supervisor. QC manager and, if in doubt, analysis by certified laboratory.
When	After training and every harvest thereafter. During the treatment. One lot out of ten (sampling size should increase or decrease depending on whether there is a problem or not).

Corrective actions:

1. Re-train farmers/staff and supervise the process,
2. Adjust concentration of bath,
3. Verify SO₂ level in shrimp if concentration was high or duration longer. Keep on hold suspect lots and investigate food safety. If unfit for human consumption, divert product for non-food use or destroy it.

Verification procedure:

See section 6.10.4.12.

Record-keeping:

See section 6.10.4.13 (also see Annex A3.III Form 13 in this appendix).

As indicated above, the HACCP plan contains three annexes (following) developed by the HACCP team to address, respectively: GAP, monitoring procedures, and forms for recording the results of monitoring and corrective actions. Drafting these annexes is a straightforward activity and is not done hereafter in detail. Instead, the table of contents of each annex is presented and some elements of each annex are provided for illustrative purposes only.

TABLE A3.1
HACCP plan for shrimp farm

Critical Control Point	Significant hazard(s)	Control measure(s) (Annex A3.I)	Critical limit(s)	Monitoring procedure(s) (Annex A3.II)			Corrective action(s)	Records (Annex A3.III)	Verification
				What	How	Who			
Shrimp farm and surroundings	Presence of pesticides in shrimp	Assessment of the risk of contamination of the farm	No contamination risk	Expert assessment	Survey of pollution sources	Designated expert	No shrimp farming in contaminated sites	Forms 1 and 13	Yearly and after each crop
		Soil and water testing	Absence of pesticides in soil and water	Laboratory or rapid field tests	Soil and water analysis	QC manager	No shrimp farming in contaminated sites	Forms 2 and 13	Laboratory tests - annually or after each crop
		Eliminate the cause of contamination by pesticides	Cause of contamination eliminated	Verification and testing	Soil and water analysis	QC manager and laboratory analysis	No shrimp farming until source of contamination is eliminated	Forms 3 and 13	Yearly or after each crop
Hatchery and grow-out	Presence of pathogenic bacteria in shrimp	Training of employees on basic hygiene and health education	All employees demonstrate a good understanding and practice of basic hygiene	Hygienic practices of employees	Supervision and verification	QC Manager	Retrain to improve practices. Otherwise restrict duties to those which do not bring unqualified workers in contact with shrimp and production	Forms 4 and 13	Weekly and at the end of training
		Application of good hygienic and sanitation practices	Hygienic and sanitary practices followed strictly	Hygienic and sanitary practices	Supervision and verification	Pond supervisors	Retrain to improve practices and assess risk of contamination due to unhygienic practices	Forms 5 and 13	Weekly and every time a deviations occurs
		Construction and maintenance of physical barriers and fences to protect from fecal contamination by livestock and domestic animals	Physical barriers and fences and their maintenance are in place	State of barriers and fences	Verification	Pond supervisors	Repair barrier or fence and assess risk when barrier or fence were not protecting ponds by checking the microbiological quality of water using method described in annex II.	Forms 6 and 13	Before a production cycle and weekly
		Establishment of a list of approved feed suppliers	The use of feed from approved suppliers only	List of approved suppliers and feed origin	Verification	QC manager	Not to use feed from non authorized supplier unless duly certified.	Forms 7 and 13	Yearly and after each crop

Table A3.1 (continued)

Critical Control Point	Significant hazard(s)	Control measure(s) (Annex A3.I)	Critical limit(s)	Monitoring procedure(s) (Annex A3.II)			Corrective action(s)	Records (Annex A3.III)	Verification
				What	How	Who			
		Proper storage of feed	Proper feed storage and local conditions	Storage local conditions	Verification	Pond supervisor	Improve storage conditions and assess risk when storage conditions were inadequate	Forms 7 and 13	Yearly and after each crop
	Presence of unauthorized veterinary drugs or high residue levels of veterinary drugs in shrimps	Only approved veterinary drugs are used	No unauthorized veterinary drug is used	Type of veterinary drug	Verification	Pond supervisor/hatchery manager	Identify concerned ponds, investigate the cause of deviation, observe withdrawal time or keep product until a full safety investigation is completed. If unfit for human consumption, divert for non food use or destroy	Forms 8 and 13	After each treatment
		Treatment supervised by a licensed professional	Only licensed professionals supervise treatment	Supervision of drug application	Verification of credentials	Pond supervisor	Investigate the cause of deviations and modify drug use practice or change practitioner. Place suspect pond(s) on hold, observe withdrawal time and undertake full good safety evaluation. If unfit for human consumption, divert product for non food use or destroy	Forms 8 and 13	After each treatment and yearly
		Strict respect of withdrawal times	30 days for ox-tetracycline	Withdrawal time	Verification or chemical analysis	Pond supervisor	Ensure withdrawal duration is respected until analyses confirm levels of drug acceptable. Otherwise, keep the product on hold until a full safety investigation can be completed. If unfit for human consumption, divert product for non food use or destroy it.	Forms 8 and 13	After each treatment and yearly

Table A3.1 (continued)

Critical Control Point	Significant hazard(s)	Control measure(s) (Annex A3.I)	Critical limit(s)	Monitoring procedure(s) (Annex A3.II)			Corrective action(s)	Records (Annex A3.III)	Verification
				What	How	Who			
Harvesting and transport	Presence of pathogenic bacteria in shrimp	Training of employees on basic hygiene and health education	All employees demonstrate a good understanding and practice of basic hygiene	Hygienic practices of employees	Supervision and verification	QC Manager	Retrain to improve practices. Otherwise restrict duties to those which do not bring unqualified workers in contact with shrimp and production	Forms 4 and 13	Weekly and at the end of training
		Application of good hygienic and sanitation practices	Hygienic and sanitary practices followed strictly	Hygienic and sanitary practices	Supervision and verification	Pond supervisors	Retrain to improve practices and assess risk of contamination due to unhygienic practices	Forms 5 and 13	Weekly and every time a deviation occurs
		Use potable water for cleaning shrimp and preparing ice	Only potable (or similar quality) water is used for cleaning shrimp and preparing ice	Water quality (e.g residual chlorine)	Rapid test	Pond supervisor	Change water source or treat the water to make it potable. Keep on hold suspect lots and investigate food safety implications. If unfit for human consumption, divert product for non food use or destroy	Forms 9 and 13	After each harvest and yearly
Presence of high residue levels of SO ₂ in shrimp		Harvest rapidly and chill shrimp quickly	Shrimp to reach + 5 °C within less than 3 hours	Ratio ice/shrimp	Verification of weights and measure temperature	Pond supervisors	Add ice or ice slurry	Forms 10 and 13	After each harvest and yearly
		Training of staff on proper metabisulphite (MBS) treatment	Only trained people apply treatment of shrimp by metabisulphite	Treatment practices of employees	Supervision and verification	Pond supervisor	Retrain farmers/staff, otherwise restrict duties of unqualified people to those which do not bring them in contact with shrimp and production	Forms 11 and 13	After each crop and yearly
		Use proper metabisulphite concentration and treatment duration: if in doubt check SO ₂ level in shrimp	MBS Conc= 1.25% Duration: 1-3 minutes SO ₂ ≤ 100ppm	Weight MBS, Water volume, Duration, Concentration of SO ₂	Verification Verification Verification Analysis (AnnexII)	Pond supervisor	Analyze SO ₂ residues in shrimp if MBS concentration was high or duration long. Keep on hold suspect lots and investigate food safety risk. If unfit for human consumption, divert product for non food use or destroy it.	Forms 12 and 13	After each crop and yearly

ANNEXES

ANNEX A3.I: GOOD AQUACULTURE PRACTICES

Company VWX has developed good aquaculture practices (GAPs) to promote the application of good hygienic, handling and sanitation practices by the employees and pond supervisors at the farm.

The company's GAPs comprise:

- protection of the farm site from pollution;
- growing-water quality;
- quality of feeds;
- proper use of veterinary drugs;
- good practices during grow out;
- good harvesting practices;
- good storage and transportation practices;
- cleaning and disinfection;
- pest-eradication programme.

Useful information to draft GAP for a shrimp farm is summarized in Section 6.10.2. Examples for elements of GAP are provided hereafter for illustrative purpose.

Proper use of veterinary drugs

- All veterinary drugs for use on the shrimp farm VWX comply with national and United States regulations and are registered with the appropriate national authority.
- Control of diseases with drugs is carried out only on the basis of an accurate diagnosis. Products are only prescribed or distributed by Dr PQR, who is authorized under national regulations.
- Veterinary drugs or medicated feeds are used according to the manufacturer's instructions, with particular attention to withdrawal periods.
- Prior to administering veterinary drugs, a system is put in place by the QC manager to monitor and record the application of the drug to ensure that the withdrawal time for the batch of treated shrimp can be verified.
- Storage and transport conditions conform to the specifications on the label.
- Records are maintained for the use of veterinary drugs.

Holding and transportation

To minimize physical damage and stress:

- holding and transportation is rapid so that shrimp are not exposed unduly to high temperatures;
- shrimp is packed in ice or immersed in ice slurry to keep temperature close to 0 °C; Table A3.2 shows the ice/shrimp weight ratio used;

TABLE A3.2
Ice/shrimp weight ratio in vehicle transport

Type of truck	Kilograms of ice to preserve 100 kg of shrimp for:				
	3 hours	6 hours	12 hours	18 hours	24 hours
Insulated	22 ⁽¹⁾ to 35 ⁽²⁾	38 to 45	40 to 65	53 to 85	65 to 105
Refrigerated	17 ⁽¹⁾ to 25 ⁽²⁾ kg of ice for 100 kg of fish regardless of the duration				

⁽¹⁾ This amount of ice is used when the outside temperature is relatively low about 15 °C.

⁽²⁾ This amount of ice is used when the outside temperature is high, about 30 °C.

- all equipment for shrimp holding and transportation is easy to clean and to disinfect, and is cleaned and disinfected regularly and as appropriate;
- shrimp is not transported with any other product;
- records for transport of shrimp are maintained to ensure full product tracing.

Pest control

Pest control is carried out by a contracting company every six months and as needed for the destruction of rats, mice, cockroaches and other pests. Pest-control chemicals are kept locked and away from the working premises to prevent any risk of contamination.

Training

- All personnel at the shrimp farm level have been made aware of their role and responsibility in protecting shrimp from contamination or deterioration. Shrimp handlers have the necessary knowledge and skills to handle shrimp hygienically and with proper care. Those who handle strong cleaning chemicals or other potentially hazardous chemicals have been instructed in safe handling techniques.
- Periodic assessment of the effectiveness of training and instruction programmes is made, as well as routine supervision and checks to ensure that procedures are being carried out effectively;
- Training programmes are routinely reviewed and updated where necessary.

ANNEX A3.II: HACCP MONITORING SYSTEM

The monitoring system of company VWX comprises:

- analyses of pesticides in soil and water samples when needed;
- detection of pathogenic bacteria in water or in shrimp;
- analysis of chlorine levels;
- analysis of veterinary drug residues;
- verification of hygiene application by employees;
- verification of GAP application by employees.

As most of the ponds of company VWX are located in remote areas with very limited access to equipped laboratories, pond supervisors have been trained in the use of simple kits for rapid and simple analyses of:

- pesticide analysis of soil and water samples;
- residual chlorine in water;
- water quality;
- SO₂ residues in shrimp and shrimp sulphite dip solutions.

These rapid analytical techniques, practical for monitoring in the field, have been officially validated by the QC manager against official methods. This validation process is repeated as often as needed and whenever a new analytical kit is introduced. Likewise, training of the pond supervisors is carried regularly to ensure proper use of the analytical kits.

The following is an example of a rapid test kit for illustrative purposes. It is important that evidence about the reliability of these or other rapid tests is obtained. Otherwise, they should be validated against officially recognized methods before use.

Example: Analysis of chloramphenicol using “Veratox for Chloramphenicol”**Test**

The test is a competitive direct ELISA (www.neogen.com) that provides concentrations in parts per billion (ppb). Free chloramphenicol in the sample and controls competes with enzyme-labelled chloramphenicol (conjugate) for the antibody-binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue colour. A microwell reader is used to yield optical densities. Control optical densities

form a standard curve, and sample optical densities are plotted against the curve to calculate the exact concentration of chloramphenicol.

Procedure

Samples must be extracted prior to testing.

1. Add 50 µl of controls and samples to the antibody-coated microwells.
2. Add 50 µl of conjugate to the wells.
3. Mix. Cover the wells and incubate for 1 hour.
4. Dump liquid from the wells.
5. Wash wells thoroughly with wash buffer.
6. Tap out water on paper towel.
7. Transfer 150 µl of substrate from a reagent boat to the wells using 12-channel pipettor. Mix. Incubate for 30 min.
8. Transfer 50 µl of stopping solution from a reagent boat to the wells.
9. Read results using a microwell reader with a 450 nm filter.

Lower limit of detection: 0.1 ppb

Range of quantitation: 0.1–5 ppb

Controls provided: 0, 0.1, 0.25, 0.5, 1 and 5 ppb

ANNEX A3.III: RECORD-KEEPING

The HACCP plan (Table A3.2) developed by company VWX refers to 13 forms to record, respectively:

1. Results of study on pesticide contamination;
2. Analysis of pesticides in soils and water;
3. Action taken to eliminate pesticide contamination;
4. Training of employees;
5. Regular control of workers' hygiene;
6. Examination and repair of fences and barriers;
7. Control of feeds;
8. Control of veterinary treatment;
9. Control of water treatment;
10. Control of icing and shrimp temperature;
11. Control of cleaning and disinfection;
12. Control of bisulphite treatment;
13. Record for corrective action.

The following are three examples of record-keeping forms provided for illustrative purposes.

Form 4 – Training of employees on hygienic practices.**Shrimp company VWX**

Name of employee	Date of hiring	Date of training	Comments
QC manager: _____		Date: _____	

Form 7 – Control of Feeds.**Shrimp company VWX**

Supplier	Quantity and lot number	Date	Visual control of feed and storage conditions	Other controls	Observation
Signature of pond supervisor: _____			Date: _____		
Signature of QC manager: _____			Date: _____		

Form 13 – Recording corrective actions.

Shrimp company VWX	
Date: _____ Lot: _____ Critical Control point: _____	
Description of the control loss (deviation):	
Description of the corrective measure:	
Description of the new situation:	
Name and signature of the supervisor:	Date:
Name and signature of the QC manager:	Date:

This technical paper compiles the state of knowledge on seafood safety and quality with the aim to provide a succinct yet comprehensive resource book to seafood quality and safety managers, including topics on emerging issues such as new pathogens, the impact of climate change on seafood safety, and the changing regulatory framework. After introductory chapters about world fish production, trade, consumption and nutrition, and about the developments in safety and quality systems, the technical paper provides a detailed review of the hazards causing public health concerns in fish and fish products, covering biological, chemical and physical hazards. This is followed by chapters on seafood spoilage and quality issues; the likely impact of climate change on seafood safety; a detailed coverage of the implementation and certification of seafood safety systems covering risk mitigation and management tools, with a detailed description of the requirements for the implementation of good hygiene practices and good manufacturing practices, the Hazard Analysis and Critical Control Points (HACCP) system, and the monitoring programmes to control biotoxins, pathogenic bacteria and viruses and chemical pollutants; a section on private labelling and certification schemes; details of the international framework covering the World Trade Organization, the Codex Alimentarius Commission, the FAO Code of Conduct for Responsible Fisheries, and the World Organisation for Animal Health; and a presentation of the regulatory frameworks governing seafood trade in the European Union (Member Organization), the United States of America, Japan, Australia and New Zealand.

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