Chapter 2

Why depurate?

On a world-wide basis, the main hazards associated with the consumption of shellfish arise from the microbiological contamination of waters in which they grow, especially when the bivalve molluscs are intended to be eaten raw. Since molluscs are filter feeders they concentrate contaminants to a much higher level than that of the surrounding seawater. Contamination with bacteria and viruses in the growing area therefore determines the processing that the shellfish need to undergo in order to remove or reduce the risks from these sources before consumption. Many of the pathogens, such as viruses causing gastroenteritis and infectious hepatitis, and the bacteria causing typhoid, are usually associated with contamination by human sewage. Others, such as the bacteria causing gastroenteritis (non-Typhi *Salmonellae* and *Campylobacter*), may be associated with either sewage or with animal faeces. The latter may contaminate shellfish-growing areas when washed off the land during periods of rain.

Some other hazards are associated with naturally occurring organisms present in the marine environment. These include infections due to pathogenic marine vibrio bacteria and biotoxins produced by some single-celled algae which can cause various forms of poisoning such as paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP) and diarrhetic shellfish poisoning (DSP).

Chemical contaminants, such as heavy metals, pesticides, organochlorides, petro-chemical substances are a potential hazard in certain areas. There is no evidence, however, in epidemiological reports or the scientific literature that illness due to the consumption of shellfish contaminated with chemical substances is a significant problem.

To identify and control the hazards, identification and monitoring of growing areas are very important. Faecal bacterial indicators such as faecal coliforms or *Escherichia coli* are used to assess the risk of the presence of bacterial and viral pathogens. The use of *E. coli* is becoming more widespread as it is considered a more specific indicator of faecal contamination. Monitoring to determine the risk associated with biotoxin presence may be based on an assessment of the presence of the algae that may produce the toxins, direct estimation of the biotoxins themselves in the shellfish, or both. Monitoring of shellfish may also be undertaken for chemical contaminants.

The risk of microbial illness arising from the consumption of shellfish harvested from waters subject to low levels of microbiological contamination may be reduced by relaying in a less-contaminated area or by depurating in tanks of clean seawater, or a combination of both. Depuration alone has a limited effect on reducing the level of viruses and marine vibrios in shellfish and is not suitable for shellfish harvested from

Table 2.1: Hazards associated with bivalve mollusc consumption		
Class of hazard		**Contaminant**
Infections	Bacteria	*Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae, Campylobacter* spp., *Listeria monocytogenes*
	Viruses	Norovirus, Hepatitis A virus
Intoxications	Chemical	Heavy metals: including Mercury (Hg), Cadmium (Cd), Lead (Pb). Organics: Dioxins, Polychlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), pesticides
	Biotoxin	Paralytic shellfish poisoning (PSP), Diarrhetic shellfish poisoning (DSP), Amnesic shellfish poisoning (ASP), Neurotoxic shellfish poisoning (NSP)

more heavily contaminated areas or areas subject to contamination by hydro-carbons, heavy metals, pesticides, or biotoxins. As currently practised, the effectiveness of the process in removing viruses and marine vibrios is limited. Table 2.1 shows the main hazards associated with the consumption of bivalve molluscs.

2.1   BIVALVE MOLLUSC-ASSOCIATED ILLNESS

Gastroenteritis associated with the consumption of bivalve molluscs has been recognised for hundreds of years. Microbes that have been implicated in bivalve mollusc-associated illness are given in Table 2.2. Many of these are related to the faecal contamination of bivalve molluscan shellfisheries. In many developed temperate countries, viral gastroenteritis due to Norovirus is the most common illness associated with the consumption of bivalve molluscan shellfish although a significant number of infections due to pathogenic vibrios, including *V. parahaemolyticus* and *V. vulnificus*, occur in the United States of America. Norovirus causes a self-limiting infection that has an incubation period of approximately 12–48 hours (average about 36 hours) and which normally lasts for about 12–60 hours (average about 48 hours) and from which people usually recover without any long-lasting after effects. The main symptoms are nausea, vomiting, abdominal cramps and diarrhoea. Although viral gastroenteritis is generally a mild illness, with a mortality rate of about 0.1 percent (most fatalities being in the very young and very old), the large numbers which occur in the community each year poses a significant illness and financial burden on countries. Most cases are due to person-to-person spread and the nature of illness reporting systems makes it difficult to estimate what proportion may be due to transmission via foods such as shellfish. It is also not clear to what extent secondary cases may occur from people being in contact with those made ill through consumption of shellfish.

In some countries, Hepatitis A is also a significant problem. For example, shellfish consumption has been estimated to be implicated in up to 70 percent of the cases of this illness in Italy and cooking of clams in restaurants and at home has been reported to be only partially effective in reducing the risk of illness. The incubation period is about 2 to 6 weeks (average about 4 weeks) and after-effects may last for several months. The main symptoms are fever, headache, nausea, vomiting, diarrhoea, abdominal pain and jaundice. Although the effects are more severe and long-lasting than with Norovirus, the fatality rate is still relatively low at approximately 0.2 percent.

The *Salmonella* spp. causing typhoid and paratyphoid fever contaminate shellfish via human faeces, including sewage, when the local population contains people excreting the bacteria (either as clinical cases or carriers). The other species that cause gastroenteritis are associated with both human and animal faeces. Shellfish-associated infections with *Salmonella* spp. used to be a significant problem in Europe and North

Table 2.2: Microbial causes of bivalve shellfish-associated illness

Microorganism	Incubation period	Duration	Principal signs and symptoms	Principal source of contamination of shellfish
Bacteria				
Salmonella typhi and *S. paratyphi*	*Typhi*: 1–3 weeks *Paratyphi*: 1-10 days Other source: 7 to 28 days, mean14 days	*Typhi*: up to 4 weeks *Paratyphi*: 2-3 weeks	Malaise, headache, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, bloody stools	Human faeces/ sewage
Other *Salmonella*	6 to 72 hours, mean18 to 36 hours	4–7 days	Abdominal pain, diarrhoea, chills, fever, nausea, vomiting, malaise	Human faeces/ sewage or animal/ bird faeces/slurry
Campylobacter	2 to 7 days	3–6 days	Diarrhoea (often bloody), severe abdominal pain, fever anorexia, malaise, headache, vomiting	Animal/bird faeces/slurry
Shigella	24 to 72 hours	5–7 days	Abdominal pain, diarrhoea, bloody & mucoid stools, fever	Human faeces/ sewage
Vibrio parahaemolyticus	2 to 48 hours, mean 12 hours	2–14 days (average 2.5)	Abdominal pain, diarrhoea, nausea, vomiting, fever, chills, headache	Marine environment
Vibrio vulnificus	16 hours mean < 24 hours	2–3 days	Malaise, chills, fever, prostration, cutaneous lesions, fatalities occur	Marine environment
Vibrio cholerae O1 and O139 serotypes	1–5 days, usually 2–3 days	2–5 days	Profuse, watery diarrhoea (rice-water stools), vomiting, abdominal pain, dehydration	Human faeces/ sewage
Vibrio cholerae non-O1/nonO139	2 to 3 days	Up to 1 week	Watery diarrhoea (varies from loose stools to cholera-like diarrhoea)	Marine environment
Viruses				
Norovirus	1–3 days mean 36 hours	20 to 72 hours	Diarrhoea, nausea, vomiting, abdominal pain, abdominal cramps	Human faeces/ sewage
Hepatitis A virus	10 to 50 days, mean 25 days	10 to 30 days 10% of infected persons will have prolonged or relapsing symptoms over a 6–9-month period	Fever, malaise, lassitude, anorexia, nausea, abdominal pain, jaundice	Human faeces/ sewage
Astrovirus[1]	1 to 2 days	48 to 72 hours	Diarrhoea, some times accompanied by one or more enteric signs or symptoms	Human faeces/ sewage

[1] Only a small number of shellfish-associated astrovirus infections have been reported.

America but occur less often now. This is partly due to general improvements in public health which have reduced the incidence of typhoid and paratyphoid in the community, and thus lessened the risk of the causative bacteria contaminating shellfish via sewage, and partly due to the effectiveness of current hygiene controls on shellfish production. Salmonella gastroenteritis associated with shellfish consumption still does occur in these countries on some occasions when members of the public gather shellfish for their own consumption and also when shellfish are sold commercially without all of the hygiene controls being adhered to. It is likely that these bacteria still cause a large number of shellfish-associated outbreaks in subtropical and tropical countries but the illness reporting systems in such countries tend to be poor and the level of the problem is difficult to ascertain. The forms of bacterial intestinal infections

caused by *Shigella* spp. and *Campylobacter* spp. have been reported as having been associated with shellfish-consumption in the United States of America but not Europe. The reason for this difference is not known.

Pathogenic *Vibrio* spp. There are a number of species of *Vibrio* that cause illness associated with the consumption of shellfish. The two of most importance in terms of numbers of infections and/or fatalities are *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Most of these vibrios occur naturally in coastal and estuarine environments and are not associated with sewage contamination. The types of *Vibrio cholerae* that casue epidemic cholera are usually associated with human faecal contamination although some strains of these types, and of those causing non-cholera gastroenteritis, may occur naturally in the marine environment. Chilling shellfish as soon as possible after harvest and maintaining low temperatures (less than or equal to 10 °C) has been shown to be important in preventing pathogenic vibrios from multiply to high levels. In areas of the world prone to such problems, controls may be put on harvest, post-harvest transport conditions, or post-harvest treatment (pasteurisation, high-pressure treatment, freezing or irradiation) during the summer months when the risk is highest.

Vibrio parahaemolyticus causes gastroenteritis. For many years it has been the most common reported cause of food-poisoning in Japan where it is associated with the consumption of raw fish and other seafood. Illness with the organism has also been reported from other parts of Asia and from the United States of America, Canada, Africa and southern Europe although imported cases can occur anywhere. Outside of Japan, infections are often associated with the consumption of raw oysters although undercooked or cross-contaminated crustacea have also been involved. Predominant symptoms are nausea, vomiting, diarrhoea, abdominal cramps and fever. The incubation period is between 4 and 96 hours (average 15) and the average length of illness is 2.5 days. Not all strains of *V. parahaemolyticus* are pathogenic and most strains found in the environment and seafood cannot cause gastro-enteritis. The pathogenicity of a strain depends on the presence of specific genes, therefore specialized molecular tests are needed to confirm that an isolate from seafood may be capable of causing illness. An international risk assessment (FAO/World Health Organization) for *V. parahaemolyticus* in oysters has been completed and the document is expected to be released soon.

Vibrio vulnificus can cause wound infections if open cuts come into contact with seawater (or surfaces) contaminated with the organism. It can also cause primary septicaemia when the organism enters the body via the intestinal tract, typically after eating contaminated oysters, and then infects the bloodstream. Both wound infections and primary septicaemia can be fatal with the mortality rate associated with the former being in the range 7 to 25 percent and with the latter being about 50 percent. *V. vulnificus* septicaemia is usually associated with pre-existing illness such as diabetes, liver or kidney disease or a problem with the immune system. The incubation period has been reported to vary from 7 hours to several days. Without rapid specific treatment, death from the illness can occur within hours of the symptoms becoming apparent. Most cases and deaths associated with this organism have been reported from the Gulf Coast of the United States of America but there have also been reports of infections from Asia. It is suspected that strains differ in their ability to cause illness but this has not been conclusively proven. Wound infections associated with the handling of finfish (including eels) have also been seen in northern Europe and Israel but no cases of oyster-associated primary septicaemia have been reported from these regions. An international risk assessment has been undertaken on *V. vulnificus* in raw oysters (FAO/WHO [2005]: http://www.fao.org/docrep/008/a0252e/a0252e00.htm).

Vibrio cholerae strains vary markedly in their characteristics – many probably cannot cause gastrointestinal infection in humans while a proportion are able to cause severe watery diarrhoea, which may be fatal and capable of epidemic or pandemic spread – the illness cholera. Others may cause a gastro-enteritis more like that caused by *Salmonella* and these are usually associated with individual cases or small outbreaks. Those strains (enterotoxigenic *V. cholerae* O1) associated with the cholera illness are usually transmitted by faecal contamination of drinking water or foodstuffs, the latter often being contaminated via rinse water, etc. There have been reports of transmission via raw or undercooked shellfish. The other pathogenic strains (*V. cholerae* non-O1) may occur naturally in the marine environment and these have been reported to be associated with the consumption of raw shellfish in the United States of America.

Shellfish-associated gastro-intestinal illness due to *Shigella* spp. and *Campylobacter* spp. has been reported from the United States of America but not from other countries – this may be due to differences in the effectiveness of laboratory detection and epidemiological reporting systems rather than geographical differences in the occurrence of such infections.

In addition to those micro-organisms that have been confirmed as having caused shellfish-associated infections or outbreaks, there are other pathogens of humans where infective forms have been detected within shellfish but where there is not currently good evidence that consuming shellfish has caused the associated illness in people. These include the protozoal parasites *Cryptosporidium*, *Giardia* and microsporidia.

Illness due to *Listeria monocytogenes* has so far only been linked to the consumption of smoked bivalves (specifically mussels) and not those consumed live or cooked without being smoked.

2.2　WHICH SPECIES NEED DEPURATION?

In general, all species of bivalve molluscs may be subjected to depuration in order to remove micro-organisms. Those most widely subjected to the process include oysters, mussels and clams (all of varying species depending on the part of the world). Some species such as cockles, scallops and razor clams pose specific challenges to depuration, for example the mobility of scallops makes them difficult to contain in baskets and to prevent them stirring up settled detritus. Ways have been found to circumvent many of these problems. While depuration may be the only mitigation strategy for those species eaten raw, such as oysters, many other species of bivalves are lightly cooked before eating and depuration will provide an additional safeguard. It has been noted that, as a result of different habits, some species that are eaten relatively well cooked in some countries may be eaten raw or only lightly cooked in others and thus the increase in international trade complicates assessment of the risk posed by individual shellfish species.
In this manual, information will be given on those species most widely depurated and for which good verification data are available. It should be noted that physiological requirements of the same species varies markedly with region and possibly also the specific location (e.g. with respect to salinity). Information on species other than those addressed in this manual may be available at the national or regional level.

2.3　LEGISLATIVE REQUIREMENTS

Current international food safety policy is to base food control on risk analysis. Risk analysis includes three elements:

- risk assessment, which is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food borne hazards;
- risk management, which is the process of weighing policy alternatives to accept, minimize or reduce assessed risks and to select and implement appropriate options; and
- risk communication is an interactive process of exchange of information and opinion on risk among risk assessors, risk managers, and other interested parties.

The *Codex Alimentarius* provides a general framework for controls in the context of international trade. The draft revised section (February 2008) of the fish and fishery products code of practice relating to live bivalve molluscs is given at Appendix 1. This includes several items pertinent to depuration, including specific recommendations for depuration in Section 7.5. The Codex Alimentarius "Proposed Draft Standard for Live Bivalve Molluscs and for Raw Bivalve Molluscs Processed for Direct Consumption or for Further Processing" is given at Appendix 2. The latter does not include any aspects specific to depuration although it does contain aspects relating to hygiene and quality of the product. The content of the code of practice needs to be supplemented to yield the detail necessary for application of a complete control system or to define good practice.

The rest of this section outlines general considerations relating to public health controls on commercial shellfish production and gives examples relating to the European Union (EU) and United States (US) systems which are both important in terms of world trade as they dictate standards which countries exporting to these markets must meet.

In the late 1800s and early 1900s, the principal identified illness problem related to the consumption of bivalve molluscan shellfish was typhoid fever. This not only resulted in large outbreaks of illness but also caused a significant number of deaths. These outbreaks eventually led to the instigation of regulatory controls in a number of countries including the United Kingdom (UK), France, Italy, United States of America and others. Methods for depuration as a means of reducing the risk of illness from shellfish consumption were developed during the late 1800s, while legislative controls in Europe and the United States of America were introduced in the 1900s.

In general, these regulatory controls have been successful in controlling sewage-associated bacterial illnesses although the reduction in shellfish-associated typhoid and paratyphoid fever in Europe and the United States of America may have been largely due to general improvements in public health reducing the presence of these organisms in sewage and thus in impacted shellfisheries.

In a number of legislative systems the requirement for depuration or other means of post-harvest reduction of microbial contamination is dictated by the classification of the harvesting area based on the extent of contamination shown by analysis of faecal indicator bacteria in a number of samples taken over a long period of time (a year or more).

In the EU, the requirements that were stipulated in the Shellfish Hygiene Directive were replaced from 1 January 2006 by similar (but not identical) requirements given in the consolidated Food Hygiene Regulations which cover all foods of animal origin. In particular, requirements to be met by food business operators are given in Regulation (EC) No. 853/2004 laying down specific hygiene rules for food of animal origin.

In the EU, classification of harvesting areas is specified in Regulation (EC) No. 854/2004 laying down specific rules for the organisation of official controls on products

Table 2.3: EU shellfish harvesting area classification criteria

Classification of harvesting areas	Microbiological standard per 100g of bivalve mollusc flesh and intravalvular fluid[1]	Treatment required
A	≤230 *E. coli*/100g of flesh and intra-valvular liquid[2]	None
B	Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three dilution Most Probable Number (MPN) test of 4 600 *E. coli*/100g of flesh and intravalvular liquid in more than 10% of samples[3]	Purification, relaying in class A area or cooking by an approved method
C	Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three dilution MPN test of 46 000 *E. coli*/100g of flesh and intravalvular liquid.	Relaying for a long period or cooking by an approved method
Prohibited	>46 000 *E. coli*/100g of flesh and intravalvular fluid[4]	Harvesting not permitted

[1] The reference method given in the Regulations is ISO TS 16649-3.

[2] By cross-reference from Regulation (EC) No 854/2004, via Regulation (EC) No 853/2004, to the Commission Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs.

[3] The 10% tolerance is allowed for a transitional period under Regulation (EC) No 1666/2006.

[4] This level is not specifically given in the Regulation but does not comply with classes A, B or C. The competent authority has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons.

of animal origin intended for human consumption. This classification is based on the levels of *Escherichia coli* in samples of shellfish. Table 2.3 shows the EU classification criteria and associated processing requirements.

The EU regulations contain few detailed stipulations regarding the way that depuration is undertaken. The principle requirement relating to the system itself is that: "Operation of the purification system must allow live bivalve molluscs rapidly to resume and to maintain filter feeding activity, to eliminate sewage contamination, not to become re-contaminated and to be able to remain alive in a suitable condition after purification for wrapping, storage and transport before being placed on the market". These aspects relate to the general principles of depuration described in Section 3 of this manual. In addition, it is stipulated that the shellfish must be continuously purified for a period sufficient to achieve compliance with the microbiological end product standard (*E. coli* ≤230/100 g; Absence of *Salmonella* in 25 g). EU Member States have tended to clarify the way that the principles of depuration and the other general criteria in the legislation are to be achieved in the application of the legislation within national approval and inspection procedures.

In the United States of America, requirements for depuration are given in Chapter XV of the Model Ordinance of the National Shellfish Sanitation Program (NSSP; US FDA 2006) (See Appendix 4). It is up to individual USA states to implement legislation following the requirements of the Model Ordinance if their industry is to be allowed to trade with other USA states. The same requirements apply to other countries wishing to trade with the United States of America. In the United States of America, classification of harvesting areas is based on the levels of faecal coliforms in samples of seawater. Table 2.4 shows the United States of America classification criteria and associated processing requirements. The depuration requirements in the NSSP are more detailed than in the EU legislation, with more specific requirements for the construction of the depuration center and operation and verification of the depuration system.

Table 2.4: US National Shellfish Sanitation Programme shellfish harvesting area classification criteria

Classification	Total coliforms (100 ml water)		Faecal coliforms (100 ml water)		Treatment required
	Geometric Mean	90 % compliance[1]	Geometric mean	90 % compliance[1]	
Approved areas	≤70	≤230	≤14	≤43	None
Restricted areas	≤700	≤2300	≤88	≤260	Purification or relaying in an approved area
Prohibited areas	No sanitary survey or conditions for approved/restricted areas not met[2]				Harvesting not permitted

[1] Values for 5-tube decimal dilution test – different 90 percent compliance values are given for the 3-tube MPN and mTEC membrane filtration tests.

[2] Aspects other than the concentration of contaminants may be used to declare an area prohibited

In Japan, the Hiroshima Prefecture is the biggest harvesting area of oysters in Japan (approximately 57 percent of the oyster production in 2004) from where 13 000 tonnes of oysters are harvested for raw consumption and 7 000 tonnes for cooking and processing. Oysters to be eaten raw must be collected from waters where the Most Probable Number of coliforms is no more than 70/100 ml of seawater. If collected from other waters, the oysters are required to be subject to depuration.

In many food safety schemes, controls relating to depuration itself cover the following requirements:

- use of clean seawater (with disinfection if the source water is not of adequate quality);
- design and construction of the system;
- operation of the system;
- demonstration of adequate performance with respect to removal of bacterial indicators;
- quality control requirements;
- end-product testing.

2.4   BIOSECURITY

The operations within a depuration plant need to be operated in conformance with the general principles of biosecurity with respect to both public and shellfish health considerations. Cleaning and disinfection procedures must prevent contamination of product within the plant from the outside while waste water and waste material from within the plant must not cause contamination of the environment, including shellfish harvesting areas, with human or shellfish pathogens.

Chapter 3

General principles of depuration

Depuration consists of placing shellfish in flowing clean seawater such that the animals resume normal pumping activity and thereby expel contaminants from their gills and intestinal tract over a period of time. The main principles are:

- The resumption of filtration activity so that contaminants are expelled
  – This involves maintenance of the correct conditions of salinity, temperature and dissolved oxygen
- The removal of contaminants
  – By settlement and/or removal by flow away from the shellfish
  – By applying the correct depuration conditions for an adequate length of time
- Avoidance of recontamination
  – By operation of a batch "all-in/all-out" system
  – By the use of clean seawater at all stages of depuration
  – By avoiding resuspension of settled expelled material
  – By cleaning the system thoroughly between batches
- Maintenance of viability and quality
  – By correct handling before, during and after depuration

3.1   RESUMPTION OF FILTRATION ACTIVITY

This requires that the animals are not subjected to undue stress prior to the depuration process. It means that the harvesting method and subsequent handling should not shock the animals too much and that they should not be exposed to temperature extremes. Once placed in the system, the physiological conditions should be such as to maximise the activity of the animals. The criteria that are relevant to this are:

Salinity
There are absolute upper and lower limits outside of which shellfish will not function properly. These limits vary with the species and origin of the shellfish. See Table 3.1 for example values. Within these limits, general advice is that the salinity used for depuration is within 20 percent of that of the harvesting area.

Table 3.1: Recommended or specified minimum salinity limits

Species		Minimum salinity (ppt)	Country
Latin name	***Common name***		
Crassostrea gigas	Pacific Oysters	20.5[1]	UK
Ostrea edulis	Flat Oysters	25.0[1]	UK
Mytilus edulis	Mussels	19.0[1]	UK
Cerastoderma edule	Cockles	20.0[1]	UK
Mercenaria mercenaria	Hard clam	20.5[1]	UK
Tapes decussatus	Native clam	20.5[1]	UK
Tapes philippinarum	Manila clam	20.5[1]	UK
Ensis spp.	Razor clams	30[1]	UK
Crassostrea iredalei	Slipper cupped oyster	17.5[2]	Philippines
–	Oysters	20	Japan[3]

[1] UK specification by the Centre for Environment Fisheries and Aquaculture Science (CEFAS) on behalf of the Food Standards Agency.

[2] Palpal-Latoc EQ, Caoile SJS and Cariaga AM 1986. Bacterial depuration of oyster (*Crassostrea iredalei* Faustino) in the Philppines, p 293-295. In: Maclean JL, Dizon LB and Hosillos (eds). The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

[3] Horoshima Prefecture Regulations.

Seawater abstracted from coastal locations that are not impacted by freshwater sources such as rivers, or stormwater discharges, should be of relatively constant salinity.

Temperature

Again, there are absolute upper and lower temperature limits outside of which shellfish will not function properly. See Table 3.2 for example values. However, temperatures at which the shellfish show physiological activity do not necessarily provide good removal of microbial contaminants.

Dissolved oxygen

Adequate levels of oxygen are required to ensure physiological activity. A minimum guide level of 50 percent saturation has been given in the past for *Ostrea edulis* and *Crassostrea gigas* (Wood, 1961) and this has since been applied more widely although formal evidence for the choice of this value is limited. In Hiroshima Prefecture, Japan, a minimum of 60 percent is specified for the depuration of oysters. The absolute amount of oxygen dissolved in water will vary with temperature (a lower concentration will be obtained at higher temperatures while the oxygen requirement of bivalves will

Table 3.2 Recommended or specified temperature limits for depuration

Latin name	Common name	Temperature °C		Country
		Lower	**Upper**	
Crassostrea gigas	Pacific oysters	8[1]	18[2]	UK
Ostrea edulis	Flat or native oysters	5[1]	15[2]	UK
Mytilus edulis	Mussels	5[1]	15[2]	UK
Cerastoderma edule	Cockles	7[1]	16[2]	UK
Mercenaria mercenaria	Hard clam	12[1]	20[2]	UK
Tapes decussatus	Native clam	12[1]	20[2]	UK
Tapes philippinarum	Manila clam	5[1]	20[2]	UK
Ensis spp.	Razor clams	10[1]	-	UK
Not specified	Oysters	10[3]	25[3]	USA
Mya arenaria	Soft clam	2[3]	20[3]	USA
Mercenaria mercenaria	Hard clam	10[3]	20[3]	USA

[1] UK specification by Cefas on behalf of the Food Standards Agency.

[2] Seafish Industry Authority recommendation.

[3] US NSSP – recommended values unless shown otherwise by process verification studies.

increase with temperature. In general, properly designed and operated systems should be capable of maintaining oxygen concentrations of at least 5 mg/l for mussels while higher concentrations are often easily achieved for other species. A limit of 5 mg/l is specified in the New Zealand Implementation Standard whereas this (or another) value may only be used as a guideline for approval of systems in some other countries. The method of aerating the seawater to provide the oxygen should not compromise other aspects of the process, e.g. adequate settlement of expelled faeces and pseudofaeces.

There may be difficulties in achieving 5mg/l in countries where the ambient temperature is significantly above 25 °C. In such cases, it will be necessary to validate that the use of lower oxygen concentrations will give consistent effective depuration at the prevailing temperatures and with the specific system design and shellfish species. It may be necessary to provide cooling in order to be able to achieve sufficient oxygen for effective depuration. However, cooling of depuration water in temperate climates needs to be undertaken with care as, although physiological activity may be maintained at lower temperatures, the efficiency of microbial removal, especially that of viruses, may be significantly reduced.

3.2   REMOVAL OF CONTAMINANTS

The primary purpose of depuration is the removal of microbial contaminants and this is largely achieved by providing the physiological conditions for the resumption of filtration activity and providing a good and an uninterrupted flow of water to allow the depurated material to be taken away from the shellfish. However, it should be noted that microbial removal, especially of viruses, is often not optimum over the whole range of conditions under which shellfish show filtration activity. In particular, in temperate climates, temperatures well above the minimum at which filtration occurs are usually necessary for removal of viruses. Also, consistent removal of marine vibrios may not be achieved under such conditions and there are concerns that increasing the temperature may increase the possibility of the proliferation of marine vibrios within a depuration system.

3.3   AVOIDANCE OF RECONTAMINATION

A primary requirement for avoiding recontamination during depuration is the operation of a batch "all-in/all-out" system, with no more shellfish being added to the system once the depuration cycle has been started. This is necessary to prevent partially depurated shellfish being recontaminated by the material excreted from freshly introduced shellfish. It also prevents settled faecal material being resuspended during the addition of further shellfish (see below).

It is necessary to use clean seawater both for the primary source of abstracted water, including relevant treatment, where necessary, and if seawater is recycled during a single depuration cycle, or re-used from one cycle to another.

It has been shown that bacterial pathogens may survive in faecal strands and may subsequently be released into the overlying water. It would be expected that survival, and thus the potential for recontamination, would be greater with viruses due to their greater survival in seawater.

An adequate flow of water within the system is necessary to ensure that depurated faeces and pseudofaeces are taken away from the shellfish. However, especially in
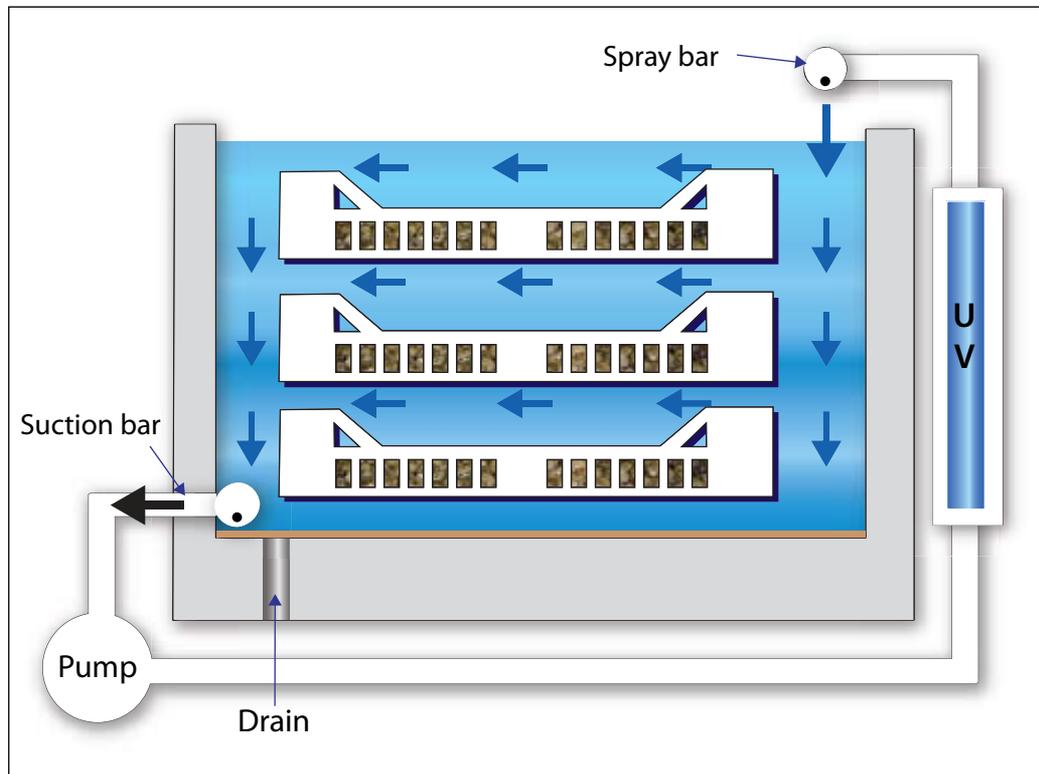
Figure 3.1: Diagram of seawater flow through a loaded tank in a recirculation system

recirculation systems, the flow must allow adequate settlement of the depurated material. If the flow is too great the strands of material will be broken up and resuspended in the seawater. Disinfection systems may not be sufficient to inactivate pathogens before they are recirculated and re-ingested. In this respect, water flow has to be a balance between that necessary for adequate activity and removal of depurated material and that which will subsequently allow settlement of the solids.

Some large systems have been designed with upward or downward flow. Upward flow is to be avoided as this will tend to keep the depurated material in suspension.

Aeration systems must also avoid resuspension of depurated material. They should not be located directly below, or impact directly upon, the shellfish themselves.

The flow of seawater through a loaded tank is shown in Figure 3.1. The flow in complete flow-through and recirculation systems is shown later in Figures 5.7 and 5.8.

Resuspension may also occur if shellfish, or the trays/baskets they are in, are removed while the water is in the system. For this reason, the water must be drained below the level of the lowest shellfish before any are removed.

3.4   MAINTENANCE OF VIABILITY AND QUALITY

Viability and quality is maintained by the following:

- proper handling and storage of the shellfish before and after depuration, avoiding both shock and excessive vibration;
- provision of adequate flow and dissolved oxygen during the depuration process;
- avoiding temperatures that are too high or too low;

- keeping the build-up of end-products such as ammonia during depuration to a minimum.

Spawning results in significantly weakened shellfish. Shellfish that have spawned should not be depurated. Those that do so in the tanks should preferably be returned to the harvesting area (if this is allowed by local regulations).

3.5 LIMITATIONS OF DEPURATION

Depuration was originally developed to remove bacterial contaminants from shellfish, primarily *S.* Typhi. In general, bacterial indicators (such as *E. coli*) and pathogens (such as *Salmonella*) of faecal origin are relatively easily removed in a properly designed and operated depuration system. Depuration has been shown to be ineffective in reducing a number of *Vibrio* species pathogenic for humans and there are concerns that, if the salinity is in the right range (e.g. 10 to 30 ppt) and the temperature is high enough (e.g. above 20 °C) an increase in the concentration of vibrios may occur during a depuration cycle.

Studies on the removal of bacteria during depuration using bivalves artificially seeded with bacterial cultures tend to show a greater degree of removal than do studies using naturally contaminated shellfish. The use of such seeding in the investigation of depuration criteria or the validation of the effectiveness of commercial systems is thus questionable.

Research undertaken in northern Europe with Pacific oysters (*Crassostrea gigas*) has shown that viruses are removed much more slowly during depuration than is *E. coli*. Even in properly designed and operated systems, approximately one-third of the starting concentration of viruses will remain after 2 days at 8 °C. At higher temperatures, e.g. from 18 to 21 °C, viruses are removed from the shellfish more quickly but, while most virus present will be removed after 5–7 days at such temperatures, some residual viral contamination may remain even when only moderately contaminated shellfish are depurated. Given that the infectious dose of these viral pathogens is thought to be low, this means that depuration cannot be regarded as a primary mitigation factor for them. However, such reductions will obviously reduce the risk of illness to some extent and therefore it is necessary to optimise the design and operation of systems for the removal of pathogens and not to target these simply at the removal of bacterial indicators such as *E. coli*. Information on the depuration of viruses from oysters is not available for warmer climates and thus it is not known whether oyster depuration in warmer climates undertaken at normal growing temperatures will be naturally more effective. Data on the depuration of mussels (*Mytilus* spp.) artificially seeded with Hepatitis A indicates that the depuration period needed for removal is also prolonged.

3.6 BIOTOXINS

Depuration in tanks is not currently considered a viable means of reducing biotoxin contamination to safe levels. The rate of depuration varies with the toxin and the bivalve species and may take from days to several months. Even for those toxins and species where more rapid removal has been demonstrated, this is often not consistent and individual animals may retain significantly higher levels of toxins than others. As with the removal of other contaminants, the rate is affected by temperature and salinity. Removal in the natural environment may be quicker than in tanks due to the availability of natural food.

3.7 CHEMICAL CONTAMINANTS

Depuration in tanks is not considered to be a practical means of removing high concentrations of heavy metal and organic chemical contaminants from bivalve molluscs. For example, polynuclear aromatic hydrocarbons (PAHs) in contaminated *Mya arenia* takes several weeks to reduce to insignificant levels.

Chapter 4

Site requirements

4.1 GENERAL LOCATION

There are several factors influencing the choice of a site to establish a depuration facility. These include:

Planning regulations
Local planning regulations may be the deciding factor as to where a depuration plant may be sited, its size and exterior design. In some countries, it is becoming more difficult to site new plants in shoreside or rural locations. This may dictate location in units on industrial estates or other urban or suburban locations.

Access to raw product
The importance of this factor in relation to location will depend on whether local shellfish are to be depurated or whether they are to be brought in from elsewhere for processing. If local shellfish are to be used, then a location reasonably close to the gathering or landing place may be preferable, depending on the availability of the other factors listed in this section.

Access to seawater
Relatively large volumes of seawater are necessary, the amount depending on the size of the facility, tank design (flow-through or recirculating) and number of cycles processed per week. An alternative approach is the addition of the correct quantity of salts to potable quality water. The quality and sources of seawater are considered in Section 4.2.

Access to transport routes for finished product
This is an important commercial consideration but the details will depend on the size of the proposed operation, distance to market and local conditions.

Waste disposal facilities
There is a need to have facilities for disposal of both liquid (used seawater and potable water) and solid waste (including broken shell). Local regulations may dictate that liquid waste from a plant discharge to the local sewerage system be treated as trade waste and be subject to a separate charge. For plants in coastal locations, it may be acceptable for used seawater to be discharged to the estuary or sea but this may not be always the case. There may be regulations covering the disposal of shellfish waste to the marine environment (as in the EU) and this will either require conditions of

disposal to be met or else the waste will have to be disposed of in some other way (e.g. to landfill).

4.2   SEAWATER QUALITY

A source of seawater of consistent good quality is a necessity for proper depuration. Water of poor quality, containing significant levels of contaminants, has the potential to cause additional contamination of the shellfish. There is also the possibility of the activity of the shellfish being inhibited by the presence of contaminants in the seawater. In addition, the composition of the seawater needs to be appropriate to the physiological requirements of the species in question and to any relevant regulatory controls. Where the locally available natural seawater is not of the required characteristics or quality, or where the depuration plant is located some distance from the sea, artificial seawater may be used instead. In a limited number of locations, saline borehole water having the required characteristics is available.

In a small number of countries, seawater is re-used from one depuration cycle to another. If this is undertaken, a higher standard of water treatment is advisable in order to remove metabolic by-products and maintain depuration efficiency. In addition, a proportion of the seawater should be replaced with new water on a regular basis – this is necessary anyway to replace water lost during cleaning of systems after each cycle. Also, the entire volume of seawater should be replaced on a regular basis. Care needs to be taken that evaporation during re-use does not result in salinities that are too high to permit effective depuration. In the UK, the re-use of seawater is permitted under specific conditions given for the individual plant and system by the central authorities. This allowance has been made to reduce the burden on industry where ready supplies of good quality seawater are not available and where adverse weather or tides intermittently prevent good quality seawater from being abstracted. However, it is generally the case that the efficiency of depuration declines with re-use and therefore it is not recommended. In many countries, it is specifically not allowed.

4.2.1   Natural seawater
In general, natural seawater for use in depuration should have the following properties:

- If it is to be subjected to disinfection prior to use: be taken from an area that at least conforms to the requirements for a production area suitable for depuration (EU class B, US Restricted);
- If it is NOT to be subjected to disinfection prior to use: be taken from an area that at least conforms to the requirements for a production area suitable for direct human consumption (EU class A, US Approved);
- Be free of chemical contaminants in such concentrations that may either interfere with the physiological functioning of the animals or, following uptake, result in the possibility of taints or human health effects;
- Be taken from an area free of significant concentrations of potentially toxic phytoplankton species or biotoxins;
- Have a salinity between 19 and 35 ppt (depending on species to be depurated and the salinity of the harvesting area); and
- Have a turbidity less than or equal to 15 NTU (Nephelometric Turbidity Units).

It is therefore implicit that source water should NOT be taken from areas that are currently closed for harvesting for regulatory purposes on the basis of microbiological, chemical or toxin events.

In New Zealand, there is a stipulated pH rate of 7.0-8.4 for the depuration process water.

The salinity, turbidity and extent of microbiological contamination may vary with tidal state and seawater should only be extracted when the salinity is in the correct range and turbidity and microbiological contamination are at a minimum. In general, salinity will be highest in estuaries on the flood, or at high tide and least on the ebb and at low tide. This effect may be greater at spring tide. In some estuaries, there may be stratification effects, where water of different salinities occurs at different depths, especially after rainfall. For this reason, inlet pipes should be located well below the surface (but preferably not directly on the seabed as this may risk the introduction of additional suspended solid material). Intakes should be protected by a grill over the end.

Stormy weather may cause the seawater to contain significantly greater amounts of sediment and it may not be possible to abstract water of the correct quality during such periods. In some areas, heavy rainfall may cause significantly lower salinities in estuaries and also cause increased amounts of sediment to be washed down from the rivers. In addition, operation of Combined Sewer or Stormwater Overflows may result in significantly greater amounts of microbiological contamination in the seawater during such periods.

4.2.2   Artificial seawater
Artificial seawater is prepared by dissolving an appropriate mix of salts in potable quality water from which chlorine has been removed (if appropriate). If carefully prepared from good quality water, it has the advantage that the initial quality is usually better than, and more consistent than, naturally occurring seawater. It may also be more convenient for use in depuration plants located away from the coast or where the local seawater quality is poor. For many species, the absence of food particles in the seawater does not seem to affect depuration efficiency. However, it should be noted that artificial seawater may not be suitable for the depuration of all species and that evidence of its efficacy for a particular species should be sought before it is used. Also, not all artificial seawater mixes on the market will successfully allow depuration. Appendix 6 includes further consideration of artificial seawater and gives recipes for use with a number of species depurated in northern Europe.

4.2.3   Saline borehole water
In some locations, the water table may contain water of the correct salinity for depuration and this provides a possible alternative source, again depending on local regulations allowing its use. Such sources may be microbiologically clean.

4.3   ACCESS TO UTILITIES AND LABOUR

As well as access to a supply of either good quality natural seawater or facilities to prepare artificial seawater of the right composition and quality, access to the following are necessary:

- an electricity supply (or adequately sized generators);
- potable quality water (should conform to the WHO recommendations for potable water quality (see appendix 5), or local regulatory requirements if these are stricter);
- distribution networks (local, national or international, as appropriate);
- waste disposal (used depuration water and solid waste from culling, etc.).