

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture
Organization of the
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World Health
Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 7

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME CODEX COMMITTEE ON FOOD HYGIENE

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PROPOSED DRAFT REVISION ON THE GUIDELINES ON THE *APPLICATION OF GENERAL PRINCIPLES OF FOOD HYGIENE TO THE CONTROL OF PATHOGENIC VIBRIO SPECIES IN SEAFOOD* (CXG 73-2010) AT STEP 4

REPORT OF THE VIRTUAL WORKING GROUP MEETING

26 February 2024

(Prepared by Japan, chair of the Virtual Working Group)

Background

1. A virtual working group (VWG) was held on 26 February 2024, chaired by Japan to discuss the proposed draft revision on the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood* (CXG 73-2010), taking into consideration comments received in response to CL 2024/09/FH.

Summary of Discussion

2. The VWG focused its discussion on paragraphs where several substantive comments were made (as included in CX/FH 24/54/8 Add.1) and agreed the following:

Introduction

3. Based on comments received it was agreed that the introduction section providing scientific background information of different *Vibrio* spp. in detail would be shortened, while retaining some crucial information (e.g., difference in virulence and susceptible population depending on the species and impact of climate change) and bearing in mind that the current introduction section reflects the discussion held when developing the original guidelines.

4. The list of *Vibrio* spp. in paragraph 2 was revised and moved to a footnote without mentioning exact numbers of pathogenic or foodborne disease-causing species.

Definition

5. The definition of “seafood” was modified to include seaweed based on reports of both contamination of seaweed with pathogenic *Vibrio* spp. and cases of vibriosis linked to consumption of seaweed.

6. The definition of “Treated” was proposed in place of “Thoroughly treated” since there was no actual difference between the two. Also, in the definition of “Partially treated”, “significantly” was put in square brackets as a concern was expressed that the word could be used in a different context in the HACCP system.” This should be further discussed at the plenary of CCFH54 so that these definitions are written to reflect what they intend to convey.

7. The definition of “clean water” was aligned to the one in the *Guidelines for the Safe Use and Reuse of Water in Food Production and Processing* (CXG100-2023).

Specific Paragraphs

Paragraph 34

8. Writing “potable or” every time clean water appears in the document should be discussed again taking into account the fact that clean water includes clean seawater which is not potable, the limited access to potable water in certain situations (e.g., onboard a vessel), and the outcome of the PWG on Annex II on Fishery Products and Annex III on Dairy Products for inclusion in the Guidelines for the Safe Use and Reuse of Water in Food Production as well as the “water fit for purpose” terminology used in *Guidelines for the Safe Use and Reuse of Water in Food Production and Processing* (CXG100-2023) .

9. The 2nd sentence was revised to read “The use of sea water taken from near a drainage or river contaminated with sewage should be avoided.” to make the text clearer.

10. It was agreed to use “possible” instead of “practicable” considering that original text of “as short as possible” was taken from the scientific recommendation, that there should be no or minimal delay to cooling, whereas “practicable” gave a notion with more flexibility.

Paragraph 63

11. 10 °C was considered sufficient to limit growth of *Vibrio* spp., but taking into account the scientific evidence for 5 °C to prevent growth of *Vibrio* spp. or to prolong shelf life of some products, 2 options were drafted for consideration at the plenary of CCFH54.

Option 1: 10°C/ to limit growth

Option 2: 5°C/ to prevent growth

12. The *Code of Practice for Fish and Fishery Products* indicates maintaining the product at temperature as close to 0°C as possible. For pathogenic *Vibrio* spp., a temperature of [**Option 1: 10 °C/Option2: 5°C**] or lower is adequate [**Option 1: to limit growth/Option 2: to prevent growth**]. In this Code, 10 °C is used as the target temperature to prevent/minimize growth of *Vibrio* spp. However, pathogenic bacteria species such as *Listeria monocytogenes*, *Clostridium botulinum* and histamine formers may also be hazards in addition to *Vibrio* spp. If this is the case, more strict temperature control, as close to 0°C as possible, should be implemented. In the case of bivalve molluscs, a different temperature control specified in the Annex would be required. The facility should be capable of controlling ambient temperature to ensure that product temperature during processing of raw seafood is maintained at a temperature of [**Option 1: 10 °C/Option 2: 5°C**] or lower.

Paragraph 100, Bullet point 3

13. Though the non-foodborne route is outside the scope of Codex, infection from wounds does occur when handling shellfish for food preparation. Considering its importance in conveying the message to consumers, it was agreed to keep the text and two options were proposed for the discussion at CCFH54:

Option 1: modify the text of bullet point 3 of paragraph 100 to make it more understandable and keep it where it stands now (under “9.4.1 Special attention to susceptible subpopulations”)

Option 2: new text would be added in the introduction section (revise paragraph 2).

Section XI

14. The title was changed to “Selection and Application of Methods for Detection and Enumeration of Pathogenic *Vibrio* Spp.” and the entire section was reorganized into 3 subsections; “11.1 Purpose of analytical testing”, “11.2 Choice of analytical method” and “11.3 Types of analytical methods.”

Other revisions

15. In addition to changes agreed during the VWG Appendix I also incorporates editorial suggestions in CX/FH 24/54/8 Add.1 that were not discussed during the VWG but were thought to be relevant by the WG Chairs and some additional editorial corrections to ensure the Appendix accurately reflects the changes that have been made to original version of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood* (CXG 73-2010).

Recommendations for the plenary of the 54th session of CCFH

16. It is recommended that CCFH54 consider Appendix I as the basis for discussion during the plenary session.

GUIDELINES ON THE APPLICATION OF GENERAL PRINCIPLES OF FOOD HYGIENE TO THE CONTROL OF PATHOGENIC *VIBRIO* SPECIES IN SEAFOOD (CXG 73-2010)

(Changes made to Appendix I of CX/FH24/54/8 are in strikethrough/ underline and colored text)

1. INTRODUCTION

1. ~~During the last few years~~ There has been an increase in reported outbreaks in some areas and cases of foodborne disease attributed to pathogenic *Vibrio* species. As a result, there have been several instances where the presence of pathogenic *Vibrio* spp. in seafood has led to a disruption in international trade. This has been particularly evident with *Vibrio parahaemolyticus* where ~~there has been~~ a series of pandemic outbreaks occurred due to the consumption of seafood, and its emergence has been observed in regions of the world where it was previously unreported. A number of *Vibrio* species are increasingly being recognized as potential human pathogens. The food safety concerns associated with these microorganisms have led to the need for specific guidance on potential risk management strategies for their control. These risk management strategies need to be developed and implemented based on the specific harvest area site characteristics such as water and ambient temperatures, salinity and water sources flowing into a harvest area. It was previously thought that the ingestion of a large number of viable cells was previously thought to be needed for pathogenic *Vibrio* spp. to survive the acidic environment of the stomach and establish an infection in the gastrointestinal tract. With the emergence of highly pathogenic strains, there is now recognition that the dose-response may be much lower depending on the individual strains and virulence profiles.

General Characteristics of Pathogenic *Vibrio* spp. associated with foodborne illness

2. Most species of genus *Vibrio* that are pathogenic to humans can cause food-borne illness. The genus *Vibrio* contains at least twelve species pathogenic to humans, ten of which can cause food-borne illness. The majority of food-borne illness is caused by *V. parahaemolyticus*, choleraogenic ~~*V. vibrio*~~ *V. cholerae* (O1, O139), or ~~*V. vibrio*~~ *V. vulnificus*¹. *V. parahaemolyticus* and *V. cholerae* are ~~solely~~ or mainly isolated from gastroenteritis cases that are attributable to the consumption of contaminated food (both species) or from the intake of contaminated water (*V. cholerae*). In contrast, *V. vulnificus* is primarily reported from extraintestinal infections (e.g. septicaemia, infected wounds, etc.) and primary septicaemia due to *V. vulnificus* infection is often associated with consumption of seafood. *V. alginoliticus*, non-choleraogenic *V. cholerae*, *V. fluvialis*, *V. furnissii*, *V. hollisae* (re-classified as *Grimontia hollisae*), *V. metschnikovii* and *V. mimicus* can also cause food-borne illness.

2 bis Non-foodborne route of infection of *V. vulnificus* is outside the scope of these guidelines, but special attention to the susceptible subpopulations handling shellfish will be needed to prevent *V. vulnificus* infections associated with injuries from knives or shells.

3. In tropical and temperate regions, these species of *Vibrio* occur naturally in marine, coastal and estuarine (brackish) environments and are most abundant in estuaries. Pathogenic *Vibrio* spp., in particular *V. cholerae*, can also be recovered from freshwater reaches of estuaries, where it can ~~also~~ be introduced by faecal contamination. *V. cholerae*, unlike most other *Vibrio* species, can survive in freshwater environments.

4. It is now possible to differentiate environmental strains of *V. cholerae* and *V. parahaemolyticus* between virulent and avirulent strains based on their ability ~~or inability~~ to produce ~~their~~ major virulence factors. The pathogenic mechanisms of *V. vulnificus* have not been clearly ~~elucidated~~ explained, and its virulence appears to be ~~multifaceted~~ multi-factorial and is not well understood, and therefore ~~all strains are considered virulent~~ it is recommended to implement measures to mitigate the risk assuming that all strains are potentially virulent need to be handled as pathogenic.

5. The following are important characteristics common to all *Vibrio* spp. *Vibrio* spp. are sensitive to low pH but can grow well at higher pHs, and thus infections caused by *Vibrio* spp. are frequently associated with low-acid foods. ~~In addition, It was previously thought that the ingestion of a large number of viable cells is~~ needed for pathogenic *Vibrio* spp. to survive the acidic environment of transition through the stomach and establish an

¹ Other *Vibrio* species that have been reported to cause food-borne illness include but not be limited to *V. alginoliticus*, non-choleraogenic *V. cholerae* (non O1/non O139 strains possessing the *ctx* gene for cholera toxin), *V. fluvialis*, *V. furnissii*, *V. -harveyii*, *V. hollisae* (re-classified as *Grimontia hollisae*), *V. metocus*, *V. metschnikovii*, *V. mimicus*, *V. paracholerae*, *V. -ponticus* and *V. tarrae*.

infection. ~~Cooking of food products readily inactivates *Vibrio* spp. even in highly contaminated products. Hygienic practices used with all food-borne pathogens will in general control the growth of pathogenic *Vibrio* spp. However, new and highly pathogenic strains of *Vibrio* spp. have emerged with a significantly lower infectious dose with 50% probability (ID₅₀). These strains also exhibited different growth characteristics compared to the *V. parahaemolyticus* strains used in the previous risk assessments².~~

6. There are, however, characteristics specific to each of the three major pathogenic species of *Vibrio* responsible for the majority of human infections, and therefore of country's ~~highest~~ public health ~~interest~~ ~~concern~~, that require attention as described below.

Vibrio parahaemolyticus

7. *V. parahaemolyticus* is considered to be part of the autochthonous micro~~biota~~ ~~flora~~ in the estuarine and coastal environments in tropical to temperate zones. ~~Seawater temperature has been reported as one of the principal environmental factors increasing the abundance of *V. parahaemolyticus* in many areas of the world. The positive effect of warming seawater temperature in spring and summer of temperate zone on the abundance of *V. parahaemolyticus* has been observed in temperate regions with low and moderate temperatures. It is also found that positive correlation for temperature to *Vibrio* levels in tropical areas where there are high fluctuations, such as macro-tidal harbours and near tidal creeks. Increased levels of *V. parahaemolyticus* are correlated with warming seawater temperatures in spring and summer for temperate regions, and are observed in macro-tidal harbours and creeks with high fluctuation temperatures for tropical regions. While *V. parahaemolyticus* typically is typically undetectable in seawater at 10°C or lower, it can be cultured from sediments throughout the year at temperatures as low as 1°C. In temperate zones, the life cycle consists of a phase of survival in winter in sediments and a phase of release with the zooplankton when the temperature of the water increases up to 14 - 19 °C. *V. parahaemolyticus* is characterized by its rapid growth in the water under favourable conditions³.~~

8. ~~The vast majority of strains isolated from patients with diarrhoea produce a thermostable direct hemolysin (TDH). It has therefore been considered that pathogenic strains possess a *tdh* gene and produce TDH, and non-pathogenic strains lack the gene and the trait. Additionally, strains that produce a TDH-related hemolysin (TRH) encoded by the *trh* gene should also be regarded as pathogenic. Although detection of *tdh*- *trh*- strains among clinical strains has been the source of debate on the pathogenic roles of *tdh* and *trh* genes, and the mode of pathogenicity is not fully understood, these genes are still the most well defined markers of pathogenicity virulence.~~

8-9. ~~Symptoms of *V. parahaemolyticus* infections include explosive watery diarrhoea (sometimes watery and bloody), nausea, vomiting, abdominal cramps and, less frequently, headache, fever and chills. Most cases are self-limiting, however, severe cases of gastroenteritis requiring hospitalization have been reported. The dose response for humans is still unclear (certain epidemiological data estimated it at 1000 cells) however more data are necessary. Incubation period ranges from 7 hours to several days, with the average being ~~26~~ 24 hours. Virulent strains are seldom detected in the environment or in foods. A low proportion of environmental or food strains, including seafoods, contain known virulence markers, while ~~they~~ virulent strains are detected as major strains from faeces of infected patients. Clinical strains possess these virulence factors because they have been isolated from cases, therefore from people exhibiting symptoms caused obviously by these strains, but environmental strains are detected by chance, one shellfish could be harbouring virulent strains and another shellfish next to it not. Zones of harvest and harvest volumes can be quite large, diluting the possibility of obtaining virulent strains. Besides, on a culture plate of TCBS, the selective medium most used, there is no mean to distinguish virulent *Vp* colonies from avirulent colonies, and both can co-exist in a shellfish and on the plate. (there also could be some competition between strains). Given this limitation in testing, non detection of virulent strains in the environment or in food does not mean there is no risk to the consumer.~~

9-10. ~~*V. parahaemolyticus* was first identified as a foodborne pathogen in Japan in the 1950s. By the late 1960s and early 1970s *V. parahaemolyticus* was recognized as a cause of diarrhoeal disease worldwide. A new *V. parahaemolyticus* clone of O3:K6 serotype emerged in Calcutta in 1996. This clone, including its serovariants, has spread throughout Asia and to the USA, elevating the status of the spread of *V. parahaemolyticus* infection to pandemic. In Asia, *V. parahaemolyticus* is a common cause of foodborne disease. In general, the outbreaks are small in scale, involving fewer than 10 cases, but can occur frequently, especially in the months with high water temperature. This pandemic *V. parahaemolyticus* has now spread to at least 5 continents. There is a suggestion~~

² FAO and WHO, 2020, Advances in science and risk assessment tools for *Vibrio parahaemolyticus* and *V. vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 35) (Section 3.2).

³ FAO and WHO, 2020, Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (Section 3.1).

that ballast discharge may be a major mechanism for global spread of pandemic *V. parahaemolyticus*, but a possibility of export/import seafood-mediated international spread cannot be ruled out. ~~While the pandemic clone ST3 has now spread, other pandemic variants have emerged, such as ST36, ST43 and ST636, and have spread rapidly and globally. In addition, most countries have seen an increase in *V. parahaemolyticus* cases associated with a large genetic diversity of *V. parahaemolyticus* strains. Some genetic modifications noted in the pandemic strains include altered nucleotide bases in the *toxR* gene, an open reading frame (ORF8) in a lysogenic filamentous phage and gene sequences in 16-kb or 23-kb chromosomal inserts specific to the pandemic clone⁴.~~

40-11. ~~From the point of controlling~~ In relation to seafood-borne *V. parahaemolyticus* illnesses, harvest and post-harvest ~~are is probably~~ the most critical stage, since it is from this point onwards that individuals ~~can actually implement measures to control *V. parahaemolyticus* can be implemented.~~ Additionally, the pre-harvest control for aquaculture is also important for managing the risks. It is also important to consider control measures at post-harvest, during processing, wet storage, and associated transport and packaging operations, and during retail. ~~in particular~~ sSetting appropriate time-temperature requirements of these control measures is important, especially time-temperature controls on post-harvest refrigeration⁵.

44-12. Foods associated with illnesses due to consumption of *V. parahaemolyticus* include for example erayfish, lobster, shrimp, fish balls, boiled surf clams, jack-knife clams, fried mackerel, mussel, tuna, seafood salad, raw oysters, clams, steamed/boiled crabmeat, scallops, squid, sea urchin, mysids, and sardines finfish (such as mackerel, tuna), crustaceans (such as prawns, crabmeat), bivalve molluscs (such as oysters, scallops), cephalopods (such as squid), echinoderms (such as sea urchin) and seaweed (such as sea grapes). These products include both raw, and partially treated⁶ and thoroughly-treated seafood products that have been substantially re cross-contaminated, for example through ~~contaminated~~ utensils, water and ice, hands, coming into contact with uncooked contaminated seafood, etc.

Vibrio cholerae

42-13. *V. cholerae* is autochthonous indigenous to fresh and brackish water environments in tropical, subtropical and temperate areas worldwide. Over 200 O serogroups have been ~~established~~ identified for *V. cholerae*. Strains belonging to O1 and O139 serotypes generally possess the *ctx* gene, ~~and produce~~ which encodes the cholera toxin (CT) and are responsible for epidemic cholera. Epidemic cholera is confined mainly to developing countries with warm climates. ~~Cholera is exclusively a human disease and human faeces from infected individuals are the primary source of infection in cholera epidemics.~~ Contamination of food production environments (including aquaculture ponds) by human faeces can indirectly introduce choleraogenic *V. cholerae* into foods. The concentration of free-living choleraogenic *V. cholerae* in the natural aquatic environment is low, but *V. cholerae* is known to attach and multiply on zooplankton such as copepods.

43-14. ~~Seven pandemics of cholera have been recorded since 1823. The first six pandemics were caused by the classical biotype strains, whereas the seventh pandemic that started in 1961 and has lasted until now, is due to *V. cholerae* O1 biotype El Tor strains.~~ Epidemic cholera can be introduced from abroad spread by infected travelers, imported foods and through the ballast water of cargo ships. Detection frequencies of choleraogenic strains of *V. cholerae* from legally imported foods ~~were are~~ very low and they have seldom been implicated in cholera outbreaks. *V. cholerae* O139 has been responsible for the outbreaks of cholera in the Bengal area ~~since 1992, and this bacterium has spread to other parts of the world through travellers.~~ The choleraogenic strains of *V. cholerae* ~~that spread to different parts of the world may persist, and some factors, such as increasing imported foods, international travelers and climate change,~~ may trigger an epidemic in the newly established environments.

44-15. Some strains belonging to the O serogroups other than O1 and O139 (referred as non-O1/non-O139) can cause food-borne diarrhoea that is milder than cholera. Recent years have seen an increase in infections associated with these particular strains, with the first outbreak reported in 2018 from the consumption of herring roe.

45-16. Outbreaks of food-borne cholera have been noted in some parts of the world quite often in the past 30 years; seafood, including bivalve molluscs, crustaceans, and ~~and~~ finfish, as well as contact with ~~and~~ surface water contact and seafood handling are most often ~~incriminated in~~ linked to food-borne cholera cases in many countries.

⁴ FAO and WHO, 2020. Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (Section 2.1).

⁵ FAO and WHO, 2016. Selection and application of methods for the detection and enumeration of human-pathogenic halophilic *Vibrio* spp. in seafood (Microbiological Risk Assessment series, No. 22) (Section 2.2).

⁶ "treated" means any vibriocidal treatment (e.g., heat treatment, high pressure.). Refer to Section 2.3 (definition for "partially treated").

While shrimp has historically been a concern for transmission of cholerae *V. cholerae* in international trade, it has not been linked to outbreaks and it is rarely found in shrimp in international trade. A strong association has been observed between continuous changes in environmental and climate-related factors, particularly water temperature and salinity, and cholera infections. However, there are several complex and multifaceted epidemiological factors that are often associated with these factors.

Vibrio vulnificus

~~16-17.~~ *V. vulnificus* can occasionally cause mild gastroenteritis in healthy individuals, but it can cause primary septicaemia in individuals with chronic pre-existing conditions, especially liver disease or alcoholism, diabetes, haemochromatosis and HIV/AIDS, following consumption of raw or partially ~~cooked~~ ~~treated~~ bivalve molluscs and other seafood. This is a serious, ~~often fatal~~, disease with one of the highest fatality rates of any known foodborne bacterial pathogen. ~~The ability to acquire iron is considered essential for virulence expression of *V. vulnificus*, but a virulence determinant has not been established and, therefore, it is not clear whether only a particular group of the strains are virulent.~~ The host factor (underlying chronic diseases) appears to be the primary determinant for *V. vulnificus* infection. The dose response for humans is ~~not known~~ ~~still unclear~~ and more data are necessary. Incubation period ranges from 7 hours to several days, with the average being ~~26~~ 24 hours. ~~The dose response for humans is not known.~~ Some virulence factors have been identified, however definitive virulence determinants have not yet been established, therefore, it is not clear whether all strains are capable of causing disease. ~~The ability to acquire iron is considered essential for virulence expression of *V. vulnificus*, and other relevant virulence factors include the capsule and the MARTX toxin (Multi Functional Autoprocessing Repeat in Toxin), also known as RtxA1 toxin, the virulence correlated gene (*vcg*) and the pilus-type IV- related gene (*pilF*)^{7,8}.~~

17. — Of the three biotypes of *V. vulnificus*, biotype 1 is generally considered to be responsible for most seafood-associated human infection and thus the term *V. vulnificus* refers to biotype 1 in this Code.

18. ~~Most of the F~~ foodborne illnesses ~~from~~ associated with *V. vulnificus* ~~is characterized by~~ are sporadic cases ~~although some outbreaks have been reported~~ and an outbreak has never been reported. ~~However, outbreaks have occurred.~~ *V. vulnificus* has been isolated from oysters, other bivalve molluscs, and other seafood worldwide.

19. Seawater temperature has been reported as one of the principal environmental factors increasing the abundance of *V. vulnificus* in many areas of the world. ~~Studies in 2008 have involved inoculation of live oysters with *V. vulnificus* and have shown that *V. vulnificus* can growth is possible~~ in oysters at least in the temperature range of 13-30°C.

~~19-20.~~ The densities of *V. vulnificus* are high in oysters at harvest when water temperatures exceed 20°C in areas where *V. vulnificus* is endemic; *V. vulnificus* multiplies in oysters at a temperature higher than 13°C. The salinity optimum for *V. vulnificus* appears to vary considerably from area to area, but highest numbers are usually found at intermediate salinities of 5 to 25 g/l (ppt: parts per thousand). Relaying oysters to high salinity waters (>32 g/l (ppt: parts per thousand) was shown to reduce *V. vulnificus* numbers by 3–4 logs (<10 per g) within 2 weeks. According to the ~~data available~~ ~~data~~, the salinity in the coastal environmental ~~salinity has played~~ an important role in *V. vulnificus* incidence and population levels. Evidence shows that salinity is negatively correlated with *V. vulnificus* concentrations⁹. ~~*V. vulnificus* is affected by salinity, where Salinity~~ levels lower than 1 ppt (parts per thousand) or higher than 30 ppt will not allow *V. vulnificus* ~~its~~ growth. Flow-through depuration with higher salinity levels (>30 ppt) may be used to reduce or eliminate *V. vulnificus* in oysters. High-salinity field or high salinity recirculating aquaculture (>30 ppt) may effectively reduce *V. vulnificus* within 21 to 30 days, although reductions vary.

FAO/WHO Risk Assessments

~~20-21.~~ A number of FAO/WHO risk assessments have been conducted. ~~The first ones were on *Vibrio vulnificus* in raw oysters and cholerae *Vibrio cholerae* O1 and O139 in warm water shrimp in international trade have been which were published in (2005)¹⁰⁻¹¹.~~ Additional risk assessments on *Vibrio parahaemolyticus* in raw oysters,

⁷ FAO and WHO, 2020, Advances in science and risk assessment tools for *Vibrio parahaemolyticus* and *V. vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 35) (Section 1.3);

⁸ FAO and WHO, 2020, Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (Section 2.2);

⁹ S.M. Rasz et. al. *Vibrio parahaemolyticus* and *Vibrio vulnificus* in South America: water, seafood and human infections, Journal of Applied Microbiology 121, 1201-1222, 2016

¹⁰ FAO and WHO, 2005, Risk assessment of *Vibrio vulnificus* in raw oysters (Microbiological Risk Assessment Series, No.8);

¹¹ FAO and WHO, 2005, Risk assessment of cholerae *Vibrio cholerae* O1 and O139 in warm water shrimp in international trade (Microbiological Risk Assessment Series, No.9);

~~in raw and undercooked finfish and in *Anadara granosa* (bloody clams) have been completed and published in 2011¹². These risk assessments constitute the basis of this Code. Finally, the FAO/WHO convened an Expert Meeting on 13-17 September 2010, and a meeting report has been recently published in 2020¹³.~~

~~22. FAO/WHO convened an Expert Meeting in 2011 that produced a Guidance document on methods for detection and enumeration of *V. parahaemolyticus* and *V. vulnificus*, including performance characteristics of the methods and the application of these methods for different end uses, ranging from harvest area monitoring, postharvest process verification, end product testing, outbreak investigation and growth studies¹⁴. The experts reviewed and updated the existing risk assessment models/tools for *V. parahaemolyticus* and *V. vulnificus* that could be used to inform a range of risk management questions in a number of geographical different regions. Experts agreed that the basic information of pathogenicity (including virulence markers), major factors relevant to the survival of *V. parahaemolyticus* and *V. vulnificus* (water temperature and salinity) and other main components used in the original models have not been changed; however, there are several new models and methods that have become available in the last decade. These risk assessments constitute the basis of this Code.~~

SECTION I – OBJECTIVES

~~24-23.~~ These Guidelines provide guidance on control of pathogenic *Vibrio* spp. in seafood, with a view towards protecting the health of consumers and ensuring fair practices in food trade. The primary purpose of these Guidelines is to highlight the key control measures that can be used to minimize the likelihood of illness arising from the presence of pathogenic *Vibrio* spp. in seafood. These Guidelines ~~also~~ provide information that will be of interest to the food business operators (FBOs), food industry, consumers, regulators, competent authorities and other interested parties.

SECTION II – SCOPE, USE AND DEFINITION

2.1 Scope

~~22-24.~~ These Guidelines cover seafood that is marketed and may be consumed in a live, raw, chilled/frozen, partially treated, or thoroughly in a treated state. It is applicable ~~to~~ across the whole food chain from primary production to ~~final~~ consumption. Bivalve molluscs are covered more thoroughly in the Annex, which is supplemental to these Guidelines.

~~23-25.~~ As major causative agents of foodborne bacterial illnesses associated with seafood, the target microbiological hazards of these Guidelines are ~~three~~ pathogenic *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus* and ~~cholerae~~ *V. cholerae*). The control measures described in these Guidelines may be applicable to other pathogenic *Vibrio* spp.

2.2 Use of the document

~~24-26.~~ These Guidelines are supplemental to, and should be used in conjunction with, the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Practice for Fish and Fishery Products* (CXC 52-2003). The application of these Guidelines by countries may require modifications and amendments, taking into account regional differences such as the prevalence of pathogenic *Vibrio* spp., air and water temperatures and salinity.

2.3 Definition

~~25-27.~~ For the purpose of these Guidelines, the following definitions apply:

Definitions of the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

Refrigeration: The lowering of product temperature to limit microbial activity.

Seafood: Fish, shellfish, ~~and~~ other aquatic invertebrates, and seaweed from marine and fresh water sources and

~~¹² FAO and WHO, 2011, Risk assessment of *Vibrio parahaemolyticus* in seafood (Microbiological Risk Assessment Series, No.16).~~

~~¹³ FAO and WHO, 2020, Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20).~~

~~¹⁴ FAO and WHO, 2016, Selection and application of methods for the detection and enumeration of human-pathogenic halophilic *Vibrio* spp. in seafood (Microbiological Risk Assessment series, No. 22).~~

their products which are intended for human consumption.

Partially treated: Any treatment intended to [significantly] reduce or limit but not completely eliminate *Vibrio* spp. in seafood. ~~As a result of partial treatment, the sensory characteristics of the raw product are lost.~~

Treated: Any treatment intended to eliminate *Vibrio* spp. in seafood.

~~Thoroughly treated: Any treatment intended to eliminate *Vibrio* spp. in seafood.~~

Clean water: Water that does not meet the criteria for potable water but does not compromise the safety of the food in the context of its use. ~~Water that does not meet the criteria for potable water but from any source where harmful microbiological contamination, substances and/or toxic plankton are not present in such quantities that may affect the safety of fish, shellfish and their products intended for human consumption.~~

SECTION III - PRIMARY PRODUCTION

3.1 Environmental hygiene control

~~26-28.~~ Refer to Section 3-48.1 of the *General Principles of Food Hygiene* (CXC 1-1969). In addition:

~~27-29.~~ Generally, pre-harvest controls are more applicable to farmed bivalve molluscs and fish than to other seafood (e.g. open-sea harvested fish). Where relevant to other seafood, pre-harvest controls should be considered for areas where the likelihood of introduction of pathogenic *Vibrio* spp. is significant and can be controlled.

~~28-30.~~ Temperature, time and salinity should be considered for controlling pathogenic *Vibrio* spp. in seafood. Where applicable, specific water temperature or salinity levels that can be used as control measures should be identified based on epidemiological and exposure studies as well as monitoring of pre-harvest pathogenic *Vibrio* levels.

~~29-31.~~ For monitoring bivalve molluscs, at harvest, refer to the Annex ~~to-of-these~~ these Guidelines.

~~30-32.~~ For seafood grown in coastal localities, especially in cholera-endemic areas, care should be taken to avoid contamination harvest of seafood contaminated with faecal cholerae *V. cholerae*. This includes contamination caused by significant environmental impacts such as flooding, ~~unregulated and~~ discharges from sewage spills.

3.2 Hygienic production of seafood sources

~~31-33.~~ Refer to Section 3-28.2 of the *General Principles of Food Hygiene* (CXC 1-1969).

3.3 Handling, storage and transport

~~32-34.~~ For the storage and handling of seafood aboard fishing vessels, [portable or] clean water should be used for seafood intended to be eaten raw or partially treated, and for preparing ice ~~for such use~~. The use of sea water taken from near ~~the seashore or from~~ a drainage outlet or river contaminated with sewage should be avoided. Seafood should be held at temperatures that minimize and/or prevent the growth of pathogenic *Vibrio* spp. after harvest, for example, in an ice-water slurry, ice or refrigeration on fishing vessels and at harvest sites. The delay between harvest and refrigeration should be as short as ~~possible~~ practicable possible.

~~33-35.~~ For on-boat-on board cooked (boiled, blanched) seafood products, ice and/or refrigeration should be used to facilitate the rapid cooling. Ice made from [potable or] clean water should be used to minimize cross-contamination.

~~34-36.~~ For the storage of live seafood products, clean water should be used to ~~minimise~~ minimize ~~initial~~ cross-contamination from the water.

~~35-37.~~ When the product is required to be washed, whether onboard the boat or at port, clean water should be used.

~~36-38.~~ During on-land transportation from harvest ~~the landing port~~ to the on-shore market and/or processing establishments, ~~in order~~ to minimize and/or prevent the growth of pathogenic *Vibrio* spp. in seafood, the time elapsed between harvest and refrigeration or freezing is critical and should be minimized. Ice can be used ~~efficiently~~ to keep seafood ~~under refrigeration~~ chilled during transportation and sale. Live fish and shellfish should be transported at the lowest temperature tolerable for the species. Covered containers should be used for transport to prevent contamination.

3.4 Cleaning, maintenance and personnel hygiene at primary production

~~37-39.~~ Refer to Section ~~3-48.4~~ of the *General Principles of Food Hygiene* (CXC 1-1969) and the *Guidelines for the Safe Use and Reuse of Water in Food Production and Processing* (CXG 100-2023).

~~38-40.~~ Refer to Section ~~7-112.1~~ of the *General Principles of Food Hygiene* (CXC 1-1969). A carrier who is excreting cholerae ~~cholerogenic~~ *V. cholerae* should not handle seafood, water, or ice for the storage of seafood, which may result in the contamination of the seafood with cholerae *V. cholerae*.

SECTION IV - ESTABLISHMENT: DESIGN ~~AND OF~~ FACILITIES ~~AND~~ EQUIPMENT

Objectives

~~39-41.~~ Equipment and facilities should be designed, constructed and laid out to minimize the potential for cross-contamination and recontamination of seafood with pathogenic *Vibrio* spp.

4.1 Location ~~and structure~~

~~40-42.~~ Refer to Section ~~4-49.1~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

4.1.1 Location of ~~E~~establishments

~~41-43.~~ Refer to Section ~~4-49.1.1~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

4.1.2 ~~Equipment~~ Design and layout of food establishment

~~42-44.~~ Refer to Section ~~4-49.1.2~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

4.2 Premises and rooms

4.2.1 Design and layout

~~43-45.~~ Refer to Section ~~4-29.1.2~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

~~44-46.~~ ~~Whenever feasible, p~~ Premises and rooms should be designed to keep raw material areas separated from finished seafood product areas. This can be accomplished in a number of ways, including linear product flow (raw materials to finished products) or physical partitions.

~~45-47.~~ ~~Where feasible, t~~ The washing room for food handling equipment used ~~in the~~ for finished product manufacturing should be physically segregated from the finished product processing area.

4.2.2 Internal structures and fittings

~~46-48.~~ Refer to Section ~~4-29.1.3~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

4.2.3 Temporary/mobile premises and vending machines

~~47-49.~~ Refer to Section ~~4-39.1.4~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

4.3 Equipment

4.3.1 General

~~48-50.~~ Refer to Section ~~4-39.3.1~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

4.3.2 Food control and monitoring equipment

~~49-51.~~ Refer to Section ~~4-39.3.2~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

~~50-52.~~ The chill room should be equipped with a calibrated thermometer.

~~4.3.3 Containers for waste and inedible substances~~

~~51.~~ Refer to Section ~~4.3.3~~ of the *Recommended International Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969).

4.4 Facilities

4.4.1 General

~~52-53.~~ Refer to Section ~~4-49.2~~ of the *General Principles of Food Hygiene* (CXC 1-1969).

~~53-54.~~ Adequate facilities should be provided for the handling and washing of products.

54-55. Suitable and adequate facilities should be provided for storage and/or production of ice.

4.4.1 Water supply

~~55.~~ An adequate supply of clean water and/or potable water should be available for handling and washing of seafood to limit the load of pathogenic *Vibrio* spp.

4.4.2 Drainage and waste disposal

56. Refer to Section 9.2.1 of the *General Principles of Food Hygiene* (CXC 1-1969).

~~56-57.~~ All drainage and waste lines should be capable of coping with peak demands.

57-58. Accumulation of solid, semi-solid or liquid wastes should be minimized to prevent contamination, because pathogenic *Vibrio* spp. may grow rapidly in these wastes under certain ~~circumstances~~ conditions.

58-59. Separate and adequate facilities should be provided to prevent contamination by offal and waste material.

4.4.3 Cleaning facilities

~~59-60.~~ Refer to Section 4.4.39.2.2 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.2.1 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

4.4.4 Personnel hygiene facilities and toilets

~~60-61.~~ Refer to Section 4.4.49.2.3 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.5.1 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

4.4.5 Temperature control

~~61-62.~~ Refer to Section 4.4.59.2.4 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 4.1 of *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

~~62-63.~~ The *Code of Practice for Fish and Fishery Products* indicates maintaining the product at temperature as close to 0°C as possible. For pathogenic *Vibrio* spp., a temperature of ~~40~~ 5°C or lower is adequate to limit growth. In this Code, ~~40~~ 5°C is used as the target temperature to prevent/minimize growth of *Vibrio* spp. However, pathogenic bacteria species such as *Listeria monocytogenes*, *Clostridium botulinum* and histamine formers may also be hazards in addition to *Vibrio* spp. ~~If this is the case, more strict~~ Temperature control, as close to 0°C as possible, should be implemented. In the case of bivalve molluscs, a different temperature control specified in the Annex would be required. The facility should be capable of controlling ambient temperature to ensure that product temperature during processing of raw seafood is maintained at a temperature of ~~40~~ 5°C or lower.

Option 1 The *Code of Practice for Fish and Fishery Products* indicates maintaining the product at temperature as close to 0°C as possible. For pathogenic *Vibrio* spp., a temperature of 10 °C or lower is adequate to limit growth. In this Code, 10 °C is used as the target temperature to prevent/minimize growth of *Vibrio* spp. However, pathogenic bacteria species such as *Listeria monocytogenes*, *Clostridium botulinum* and histamine formers may also be hazards in addition to *Vibrio* spp. Temperature control, as close to 0°C as possible, should be implemented. In the case of bivalve molluscs, a different temperature control specified in the Annex would be required. The facility should be capable of controlling ambient temperature to ensure that product temperature during processing of raw seafood is maintained at a temperature of 10 °C or lower.

Option 2 The *Code of Practice for Fish and Fishery Products* indicates maintaining the product at temperature as close to 0°C as possible. For pathogenic *Vibrio* spp., a temperature of 5 °C or lower is adequate to prevent growth. In this Code, 10 °C is used as the target temperature to prevent/minimize growth of *Vibrio* spp. However, pathogenic bacteria species such as *Listeria monocytogenes*, *Clostridium botulinum* and histamine formers may also be hazards in addition to *Vibrio* spp. Temperature control, as close to 0°C as possible, should be implemented. In the case of bivalve molluscs, a different temperature control specified in the Annex would be required. The facility should be capable of controlling ambient temperature to ensure that product temperature during processing of raw seafood is maintained at a temperature of 5 °C or lower.

4.4.6 Air quality and ventilation

~~63-64.~~ Refer to Section 4.4.69.2.5 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.2.2 of *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

4.4.7 Lighting

~~64-65.~~ Refer to Section 4.4.79.2.6 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.2.3 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

4.4.8 Storage

~~65-66.~~ Refer to Section 4.4.89.2.7 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.2.2 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

SECTION V - CONTROL OF OPERATION

5.1 ~~Control of food hazards~~ Description of products and process

~~66-67.~~ Refer to Section 5.4.13.1 of the *General Principles of Food Hygiene* (CXC 1-1969).

5.2 ~~Key aspects of hygiene control systems~~ Good Hygiene Practices (GHPs)

5.2.1 Time and temperature control

~~67-68.~~ Refer to Section 4.1 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003). Time and temperature are the most important factors affecting the rate of growth of pathogenic *Vibrio* spp. in seafood. At~~During~~ each processing step, ~~in processing~~ the temperature of the product should be controlled and monitored via calibrated thermometers.

5.2.2 Specific process steps

5.2.2.1 Washing and processing

~~68-69.~~ Clean water at low temperature should be used for washing and processing whole seafood ~~at processing establishments~~. However, the eviscerated cavity of fish and other edible parts of seafood intended for raw consumption (e.g., preparation of sashimi) should be thoroughly washed with potable cold running water.

5.2.2.2 Cooking

~~69-70.~~ Time and temperature should be determined for each cooking operation to ensure the inactivation and elimination of pathogenic *Vibrio* spp.

~~70-71.~~ Any cooling in water ~~After cooking and or blanching,~~ should involve potable water ~~should be used for cooling.~~

5.2.2.3 Food processing practices

~~71-72.~~ Food processing practices ~~(e.g., acidification to pH below 4.8, salting to a sodium chloride concentration of more than 10% for *V. parahaemolyticus*, food preservatives and/or water activity less than 0.94) can be used to minimize the growth and possibly reduce the levels of pathogenic *Vibrio* spp. in seafood.~~ should be used to minimize the growth or reduce the level of the pathogenic *Vibrio* spp. in seafood. Food business operators can choose some of these apply appropriate interventions depending on their actual situation. Examples of these interventions are:

- Minimizing the growth
 - acidification to pH below 4.8;
 - adding permitted food preservatives which have efficacy in ~~reducing or~~ preventing the growth of *Vibrio* spp.
- Reducing the level
 - salting to a sodium chloride concentration of more than 10% ~~for to control~~ *V. parahaemolyticus*;
 - adding permitted food preservatives which have efficacy in reducing the level of *Vibrio* spp.
 - ~~exposing oysters or other~~ seafood to ionising energy, e.g., gamma rays, machine-generated electrons or X-rays.
 - hydrostatic compression in the range of 14,500 to 145,000 pound per square inch (100 to 1,000 megapascal (MPa));

- depuration under optimal conditions, e.g., at a temperature of 12.5°C and stocking density of two oysters/L of artificial seawater for 5 days, and/or water activity less than 0.94 and high salinity (30 ppt); and
- cryogenic individual quick freezing (IQF) involving the use of cryogenic or blast freezing technology to rapidly lower the product temperature below freezing.

The use and approval of these technologies should be done in accordance with the regulations/standards of the country where the products would be sold

73. Any practice, or combination of practices selected to reduce/inactivate pathogenic *Vibrio* spp. in seafood or control/minimize the growth of pathogenic *Vibrio* spp. should be adequately validated to ensure that the process is effective. Such validation should be performed according to the Guidelines for the Validation of the Food Safety Control Measures (CXG 69-2008).

~~72-7374.~~ For example, When Freezing could be is used to reduce the level or prevent the growth of pathogenic *Vibrio* spp. in seafood, consideration should be given to the sensitivity of pathogens to freezing. For example, *V. parahaemolyticus* and *V. vulnificus* are especially sensitive to colder temperatures. To reduce *V. parahaemolyticus* and/or *V. vulnificus* to nondetectable levels, the IQF process should be followed by a period of frozen storage, which may vary depending on organism. ~~It is needed to consider~~ When freezing, the following should be considered: the freezing temperature, length of the time, initial microbial load, and the rate of temperature decreaseing while freezing^{15,16}.

~~73. Several possible technologies such as high pressure, mild heating, freezing and extended storage, have been reported to inactivate *Vibrio* spp.⁵. The use of these technologies should be done in accordance with the legislation of the country of retail sale.~~

~~74. Any practice or combination of practices selected to reduce/inactivate pathogenic *Vibrio* spp. in seafood or control/minimize the growth of pathogenic *Vibrio* spp. should be adequately validated to ensure that the process is effective. Such validation should be performed according to the Guidelines for the Validation of the Food Safety Control Measures (CXG 69-2008).~~

75. The food processing practices should be closely monitored and verified to ensure that pathogenic *Vibrio* spp. are controlled and/or reduced as intended.

5.2.2.4 Storage

76. Seafood intended for raw consumption should be stored in shallow layers and surrounded by sufficient quantities of finely crushed ice or with a mixture of ice and [potable or] clean water. Live fish and shellfish should be stored at the lowest temperature tolerable for the species (Refer to Section 9 of the *Code of Practice for Fish and Fisher Products* (CXC 52-2003)).

77. Over-stacking and/or over-filling of containers should be avoided to allow cold air to circulate adequately.

5.2.3 Microbiological ~~and other~~ specifications

78. Refer to Section ~~5-2-3~~13.2.3 of the *General Principles of Food Hygiene* (CXC 1-1969) and the *Principles for the Establishment and Application of Microbiological Criteria for Foods* (CXG 21-1997).

5.2.4 Microbiological ~~cross~~-contamination

79. Refer to Section ~~5-2-4~~13.2.4 of the *General Principles of Food Hygiene* (CXC 1-1969) and Sections 3.2.2 and 3.3.2 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

5.2.5 Physical and chemical contamination

⁵ Section 3.2 of the Risk assessment of *Vibrio vulnificus* in raw oysters (FAO/WHO Microbiological Risk Assessment Series 8); FAO and WHO, 20XX. Risk assessment of *Vibrio parahaemolyticus* in seafood. Microbiological Risk Assessment Series, No. XX (In press); FAO and WHO, 2005. Risk assessment of choleraenic *Vibrio cholerae* O1 and O139 in warm-water shrimp in international trade. Microbiological Risk Assessment Series, No.9.

¹⁵ FAO and WHO, 2020, Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (See section 3.5)

¹⁶ FAO and WHO, 2020, Advances in science and risk assessment tools for *Vibrio parahaemolyticus* and *V. vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 35) (Section 3.4).

80. Refer to Section ~~5.2.5~~13.2.5 and 13.2.6 the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.2.2 and 3.3.2 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

5.3 Incoming material requirements

81. Refer to Section ~~5.3~~13.2.8 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section ~~8.5.19.5.1~~ of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

5.4 Packaging

82. Refer to Section ~~5.4~~13.2.9 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section ~~8.5.19.5.2~~ of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

5.5 Water

5.5.1 In contact with food

83. Refer to Section ~~5.5.4~~13.3 of the *General Principles of Food Hygiene* (CXC 1-1969) and *Guidelines for the Safe Use and Reuse of Water in Food Production and Processing* (CXG 100-2023) except cases specified within this Code where clean water could be used.

84. Coastal seawaters used at landing docks and at markets have been shown to be occasionally contaminated with high level of pathogenic *V. parahaemolyticus*. Therefore, only clean/potable waters should be used in the post-harvest stage.

5.5.2 As an ingredient

85. Refer to Section ~~5.5.2~~13.3 of the *General Principles of Food Hygiene* (CXC 1-1969) and *Guidelines for the Safe Use and Reuse of Water in Food Production and Processing* (CXG 100-2023).

5.5.3 Ice and steam

86. Refer to Section ~~5.5.3~~13.3 of the *General Principles of Food Hygiene* (CXC 1-1969) and *Guidelines for the Safe Use and Reuse of Water in Food Production and Processing* (CXG 100-2023).

5.6 Management and supervision

~~87. Refer to Section 5.6 of the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1-1969).~~

5.76 Documentation and records

~~88-87.~~ Refer to Section ~~5.7~~13.4 of the *General Principles of Food Hygiene* (CXC 1-1969).

88. Records should show information regarding the control measures being monitored, for example time and temperature, at key process steps for mitigation of pathogenic *Vibrio*.

5.78 Recall procedures – removal from the market of unsafe food

89. Refer to Section ~~5.8~~13.5 of the *General Principles of Food Hygiene* (CXC 1-1969).

SECTION VI - ESTABLISHMENT MAINTENANCE, CLEANING AND DISINFECTION, AND SANITATION - PEST CONTROL

90. Refer to Section ~~6~~11 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.4 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

SECTION VII - ~~ESTABLISHMENT~~: PERSONAL HYGIENE

91. Refer to Section ~~7~~12 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.5 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

SECTION VIII – TRANSPORTATION

92. Refer to Section 8.15 of the *General Principles of Food Hygiene* (CXC 1-1969) and Sections 3.6 and 4.7.21 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

93. Transportation is an integral step in the food chain and should be carried out using suitable means, and temperature during this period should be as low as possible and should be controlled, monitored and recorded where appropriate.

SECTION IX - PRODUCT INFORMATION AND CONSUMER AWARENESS

9.1 Lot identification and traceability

94. Refer to Section 9.14.1 of the *General Principles of Food Hygiene* (CXC 1-1969).

9.2 Product information

95. Refer to Section 9.214.2 of the *General Principles of Food Hygiene* (CXC 1-1969).

9.3 Product Labelling

96. Refer to the *General Standard for the Labelling of Prepackaged Foods* (CXSODEX-STAN 1-1985). Where appropriate, product labels should include information on safe handling practices and storage recommendations.

97. In addition, countries should give consideration to labelling of unpackaged live or raw seafood, so that consumers are adequately informed with respect to the safety and true nature (alive or not alive) of these products. In particular, seafood that is at a high risk of being contaminated with pathogenic *Vibrio* spp., should be labelled to alert at-risk consumers to avoid raw consumption or cook these products, in line with the legislation in the countries where these products are retailed or sold. Any treatment (e.g., heat treatment) and storage condition, that is to be applied to the product should be mentioned in the labelling if consumers would be misled by its omission.

9.4 Consumer education

98. Since each country has specific food habits, communication and education programs pertaining to pathogenic *Vibrio* spp. are most effective when established by individual governments.

99. Programs should be directed at consumers:

- To educate them on household practices and behaviours, as indicated in Five Keys to Safer Food (WHO), that would specifically to keep the numbers of pathogenic *Vibrio* spp. that may be present in foods, to as low a level as possible and to minimize the potential of cross-contamination from seafood, to hands of via from food handlers, and then from hands to other foods, or from from seafood to utensils (e.g., cutting board), and then from utensils to other foods by:

- keeping seafood cold to minimize and/or prevent the growth of pathogenic *Vibrio* spp.;
- keeping refrigerator temperatures as low as practical;
- using thermometers inside home refrigerators, ice chests or other storage containers;
- preparing, cooking and/or consuming seafood immediately after removing them from the refrigerator;
- promptly refrigerating leftover seafood in shallow containers that encourage for rapid and even cooling;
- washing and disinfecting hands, utensils and equipments whenever raw seafood is handled; and
- using separateing utensils and equipment used for raw and cooked seafood, from these use for finished product, where appropriate.

- To help them make informed choices about the purchase, storage, shelf-life labelling and appropriate consumption of certain raw seafood that have been identified in relevant risk assessment and other studies, taking into consideration the specific regional conditions and consumption habits.

9.4.1 Special attention to susceptible subpopulations

100. Liver disease is a prominent risk factor for human infection with pathogenic *Vibrio* spp., especially *V. vulnificus*. Additional risk factors include diabetes, haemochromatosis and HIV/AIDS¹⁷ Subpopulations with increased susceptibility should follow the advice below:

- Avoid the consumption of raw or partially treated seafood; and
- Cook seafood thoroughly before consumption.
- Handle shellfish safely to avoid *V. vulnificus* infections associated with injuries from knives and or shells.

SECTION X – TRAINING AND COMPETENCE

10.1 Awareness and responsibilities

101. Refer to Section 10.1 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.8 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

102. Industry (fishermen, primary producers, manufacturers, distributors, retailers and food service/institutional establishments) and trade associations play an important role in providing specific instructions and/or training to employees for the control of pathogenic *Vibrio* spp. Special consideration should be given to possible differences in prevalence of pathogenic *Vibrio* spp. in the harvesting areas and various fishing techniques.

10.2 Training programmes

103. Personnel involved in the primary production, harvesting, processing and handling of seafood should have appropriate training for the tasks they are performing. This may include:

- The nature of pathogenic *Vibrio* spp., namely *V. parahaemolyticus*, ~~cholerae~~ *V. cholerae* and *V. vulnificus*, their harbourage sites, and their resistance to various environmental conditions to be able to conduct a suitable hazard analysis for their products;
- Prevention and Control measures for reducing the risk of pathogenic *Vibrio* spp. associated with seafood during harvesting, processing, distribution, marketing, use and storage, for preventing cross-contamination and minimizing the growth of pathogenic *Vibrio* spp.; and
- The means for verifying effectiveness of control programs, including sampling and analytical techniques.

10.3 Instruction and supervision

104. Refer to Section 10.3 of the *General Principles of Food Hygiene* (CXC 1-1969).

10.4 Refresher training

105. Refer to Section 10.4 of the *General Principles of Food Hygiene* (CXC 1-1969) and Section 3.8 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).

SECTION XI – LABORATORY ANALYSIS CRITERIA FOR DETECTION AND ENUMERATION OF PATHOGENIC VIBRIO SPP.

~~106. The choice of analytical method should reflect both the type of sample to be tested and the purpose for which the data collected will be used. The purpose of analysis for bacterial foodborne pathogens, including pathogenic *Vibrio* spp., can be divided into the following categories:~~

- ~~• harvest area monitoring;~~
- ~~• post-harvest process verification and end-product monitoring;~~
- ~~• public health investigations~~

~~107. The target of analysis for pathogenic *Vibrio* spp. are seafood and environmental samples (water, soil, sewage) from habitats or harvest area, etc.~~

~~108. Although it differs depending on the end uses, the purpose of the analysis is to determine whether the product conforms to the standards of the country or region, to demonstrate the reduction of pathogenic *Vibrio* spp.~~

¹⁷ FAO and WHO, 2005, Risk assessment of *Vibrio vulnificus* in raw oysters (Microbiological Risk Assessment Series, No.8).

using post-harvest process, to continuously investigate the environment, and to conduct risk assessment at the national, regional, or global level.

109. The analysis methods include direct plating, selective enrichment, most probable number (MPN) assay, robe hybridization on plate assay, conventional PCR, quantitative PCR, Loop-mediated isothermal amplification assay, etc. Useful guidance has been provided for the selection of appropriate analytical method depending on the potential end use of the obtained data¹³.

110. It is possible to genetically analyze the characteristics of bacterial strains between food and clinical isolates, and investigate the possibility that the strains are the same.

111. Research on the virulence factors and virulence related genes of *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* is ongoing, and these genes can be used as PCR targets to assess the pathogenicity of the bacteria strains.

SECTION XI- SELECTION AND APPLICATION OF METHODS FOR DETECTION AND ENUMERATION OF PATHOGENIC *VIBRIO* SPP.

11.1 Purpose of analytical testing

106. The purpose of analytical testing for bacterial foodborne pathogens, including pathogenic *Vibrio* spp, can be divided into the following categories:

- harvest area monitoring (to assist with establishing harvest area *Vibrio* spp management plans, where *Vibrio* abundance can be linked to specific harvest area water temperatures, salinity or other parameters, as determined by the assessment of the area)
- post-harvest process verification including end product monitoring (as part of a quality assurance program)
- public health investigation following an incident.

Sampling plans and design must consider the purpose for which it will be used.

11.2 Choice of analytical method

107. The choice of analytical method should reflect:

- the type of sample to be tested;
- the purpose for which the data collected will be used (as per paragraph 106);
- the desired level of sensitivity and test frequency
- whether a presence/absence or quantitative test is more appropriate
- whether detections of sub-populations (e.g. virulence markers) is necessary
- whether typing (e.g serotype) of pathogenic strains is required

11.3 Types of analytical methods

108. Additional guidance on selecting analytical methods is available in FAO and WHO, 2016, Selection and application of methods for the detection and enumeration of human pathogenic halophilic *Vibrio* spp. In seafood (Microbiological Risk Assessment series No. 22) and 2021, Advances in science and risk assessment tools for *Vibrio parahaemolyticus* and *V. vulnificus* associated with seafood (Section 3.5) (MRA series No.35)

109. Research on virulence factors and virulence related genes of *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* is ongoing, and these genes can be used as PCR targets to assess the pathogenicity of the bacterial strains.

ANNEX ON THE CONTROL MEASURES FOR *VIBRIO PARAHAEMOLYTICUS* AND *VIBRIO VULNIFICUS* IN BIVALVE MOLLUSCS¹⁸

INTRODUCTION

1. Bivalve molluscs are a well-documented vehicle for transmission of illnesses caused by *Vibrio* spp., especially *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Bivalve molluscs are unique in that they are harvested, handled and consumed differently from most other seafood products and therefore present unique risks and control options. They are inherently riskier than other seafood because of their filter feeding activity that concentrates pathogens present in the water. They are often consumed live and raw or after insufficient cooking. According to FAO/WHO risk assessments for both of these pathogens in many countries, bivalve molluscs are often kept alive out of water for days after harvest at ambient temperatures which allows the growth of *V. parahaemolyticus* and *V. vulnificus*.

SECTION I – OBJECTIVES

2. The purpose of this Annex is to provide guidance on control measures that minimize the risk arising from the presence of pathogenic *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs. It deals with the means to minimize and/or prevent the introduction/contamination and/or the growth of these pathogens, and adequate partial treatment¹⁸ of bivalve molluscs before consumption. Control measures required for these pathogens are similar but not the same to the extent that they have different characteristics ~~for~~ the growth and survival. The control measures outlined in this Annex reflects these differences, where they exist. This Annex further provides information that may be of interest to ~~regulatory-competent~~ authorities, ~~the food industry~~ FBOs, consumers, and other interested parties.

SECTION II – SCOPE, DEFINITION AND USE OF THE DOCUMENT

2.1 Scope

3. This Annex covers bivalve molluscs that are intended for consumption in a live, raw, or partially treated state. Bivalve molluscs (e.g., clams, mussels and oysters) consumed after a vibriocidal treatment are not covered in this Annex, noting that the control measures presented in the main documents are sufficient to control the safety of these products. The target microbiological hazards of this Annex are only pathogenic *V. parahaemolyticus* and *V. vulnificus*.

4. This Annex highlights the key control measures that influence the introduction/contamination of and minimize levels of *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs and thus the risk of foodborne diseases caused by these pathogens.

5. This Annex provides guidance applicable throughout the food chain, from primary production through to final consumption of bivalve molluscs and particular guidance on post-harvest processing. Controls measures presented in Part I apply to live and raw bivalve molluscs (including those that receive post-harvest processing), while those in Part II apply to bivalve molluscs consumed after partial treatment¹⁹.

2.2 Definitions

6. For the purpose of this Annex, the following definitions apply:

Definitions contained in the *General Principles of Food Hygiene* (CXC 1-1969), the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*; and live and raw bivalve molluscs production definitions defined in the *Standard for Live and Raw Bivalve Molluscs* (CXSODEX STAN 292-2008).

Post-harvest processing: processes (e.g., high pressure and mild heating) or treatments (e.g., freezing) intended to significantly reduce or limit but not necessarily completely eliminate *V. parahaemolyticus* and *V. vulnificus* while essentially retaining the sensory characteristics of live bivalve molluscs (Section 7.7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003)).

2.3 Use of the document

7. This Annex is supplemental to and should be used in conjunction with, the *General Principles of Food*

¹⁸ Phylum Mollusca: Class Bivalvia

¹⁸ Including cooking.

¹⁹ Risk assessment of *V. parahaemolyticus* in *Anadara granosa* (bloody clams)

Hygiene (CXC 1-1969), the *Code of Practice for Fish and Fishery Products* (CXC 52-2003), Hygiene section of the *Standard for Live and Raw Bivalve Molluscs* (CXSODEX-STAN 292-2008) and the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*. This Annex may require modifications and amendments in use, taking into account such factors as regional differences in the prevalence of pathogenic strains of *V. parahaemolyticus* and *V. vulnificus* and the epidemiological data, including the susceptibility of the population.

PART I: BIVALVE MOLLUSCS CONSUMED LIVE AND RAW

SECTION III - PRIMARY PRODUCTION

3.1 Environmental hygiene-control

8. Refer to Section 3.48.1 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section 3.1 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

9. The control measures described in this section generally apply to pre-harvest environmental conditions and practices during and immediately following harvest, typically while under the control of the harvester. Effective control measures for *V. parahaemolyticus* and *V. vulnificus* will typically require an evaluation in terms of the risk associated with environmental factors in the harvesting area and harvesting practices based on epidemiology and environmental conditions (i.e., air and water temperature and salinity). An important element in estimating risk is that *V. parahaemolyticus* grows faster and at colder temperatures than *V. vulnificus*. Predictive tools using these environmental monitoring parameters and growth rates as inputs have been elaborated based on the FAO/WHO risk assessments and, when validated, may be used to estimate corresponding *V. parahaemolyticus* and *V. vulnificus* levels and risk. The predictive ability can be improved by incorporating local data and considering additional factors such as hydrodynamic effects (occurrence of tidal waves, rainfall) and sunlight. In addition to seawater temperature and salinity, some additional abiotic and biotic factors have been identified modulating the presence and abundance of *V. vulnificus* and *V. parahaemolyticus* in coastal water around the world. However, the effects of these variables are not conclusive and, in some cases, have been reported in a particular study affecting a specific area. In addition, the presence of chlorophyll, turbidity, high water temperature, and the bacteriophages are known to be related to *Vibrio* abundance²⁰.

10. In cases where predictive models are used to estimate the concentration and risks of pathogenic *Vibrio* spp. in seawater and/or bivalve molluscs based on air and water temperatures and/or salinity, their accuracy would be enhanced by incorporating local data on levels of total and pathogenic *V. parahaemolyticus* and *V. vulnificus* and growth in local bivalve species. Factors such as hydrodynamic effects (e.g., currents, tides, hurricanes and rainfall) and sunlight influence the levels of *Vibrio* spp. ~~JEMRA20 4.5.1.2 states that the *V. parahaemolyticus* prediction model as it currently exists is a linear model and therefore may be useful to estimate relative change in risk (percent reduction in risk) for different countries with more virulent strains, provided that the ranges of doses in that country are much less than the range of virulent strains. in risk (percent reduction in risk) for different countries with more virulent strains, provided that the ranges of doses in that country are much less than the ID50 for the more virulent strain (i.e., in the linear range of the dose response relationship). For *V. vulnificus*, the FAO/WHO *V. vulnificus* calculation tool is unlikely to be applicable to a wider area outside the U.S. because of different environmental, fishing, and post-harvest parameters. More importantly, however, the basis for the dose-response relationship is derived from rice epidemiological data coupled with estimated exposure levels. It has also been shown that certain shellfish species may influence risk estimates. The dose response model used in the predictive tool may need modifications based on epidemiology, as regional differences exist in the prevalence of pathogenic strains of *V. parahaemolyticus* and *V. vulnificus* including attack rate relative to exposure to *V. parahaemolyticus* strains occurred in those areas concerned²¹.~~

11. Monitoring of bivalve molluscs ~~at harvest~~ for the levels of total *V. vulnificus* and total and pathogenic *V. parahaemolyticus* should be conducted periodically overtime for lengthy period to determine the regional and seasonal variation. Prevalence of pathogenic strains of *V. parahaemolyticus* and *V. vulnificus* and the epidemiological data, including the susceptibility of the population, should be considered²². This information and some factors articulated in paragraph 15 are useful for model inputs and evaluation of model outputs as well as for the and application of appropriate controls.

12. ~~Additionally, there are some indications that *Vibrio* spp. can be introduced into a harvest area through the release of ballast water. Therefore, the impact of ballast discharge in or around the harvesting areas should be~~

²⁰ FAO and WHO, 2020, the Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (Section 3.5).

²¹ FAO and WHO, 2020, the Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (Section 4.5.1.2).

²² As an example, pandemic *V. parahaemolyticus* may require more stringent controls than other strains of pathogenic *V. parahaemolyticus* because epidemiological evidence indicates higher attack rates.

controlled ~~regarding~~ due to potential for contamination by a range of hazards, including the presence of *Vibrio* spp., especially in areas that are in close proximity to international shipping lanes.

13. Factors to be considered in determining the need for controls in a given harvest area include:
- The number of sporadic illnesses and outbreaks of *V. parahaemolyticus* and *V. vulnificus* associated with bivalve molluscs harvested from a distinct hydrographic area, and whether these illnesses are indicative of an annual reoccurrence or an unusual increase of *Vibrio* spp. illnesses is reported;
 - Water temperatures representative of harvesting conditions. Water temperatures below 15°C²³ for *V. parahaemolyticus* and below 20°C for *V. vulnificus* have generally not been historically associated with illnesses;
 - Time period to first refrigeration and post-harvest air temperatures above the minimum growth temperatures for *V. parahaemolyticus* (10°C) and *V. vulnificus* (13°C), which may increase risk regardless of harvest water temperature;
 - Harvest practices that allow radiant solar heating to raise temperatures of bivalve molluscs to temperatures above ambient air temperatures prior to harvest (i.e., intertidal harvest) and exposure time;
 - Salinity ranges and optima are different for *V. parahaemolyticus* and *V. vulnificus*. Environmental and epidemiological data indicate that there are low *V. parahaemolyticus* and *V. vulnificus* levels and few cases of illnesses ~~are~~ associated with bivalve molluscs when salinity exceeds 35 ppt (g/l) and 30 ppt (g/l), respectively. The effects of salinity and temperature on abundance of *Vibrio* differ depending on the range of fluctuations in water temperature and salinity throughout the year²⁴.
14. The competent authority should inform food business operators of the control measures contained in Sections 3.2 (Hygienic production ~~of food sources~~), 3.3 (Handling, storage and transportation) and 5.1 (~~Control of food hazards~~ Description of products and process) and 5.2 (~~Key aspects of hygiene control systems~~ GHPs) of this Annex when at least:
- Levels of *V. parahaemolyticus* and/or *V. vulnificus*, or environmental parameters exceed testing/monitoring criteria that are based on risk assessment, if applicable.
 - Environmental conditions on harvesting areas could represent a risk for *V. parahaemolyticus* and/or *V. vulnificus*, for example seawater average temperature.
 - An unusual increase of *Vibrio* spp. illnesses is reported.
- 15 The activities described in this section should be implemented by producers in cooperation with the competent regulatory authority having jurisdiction.

3.2 Hygienic production ~~of food sources~~

16. Pre-harvest and harvest measures should be applied as necessary based upon the factors identified in Section 3.1 above, such as:
- Restrict harvest or otherwise prevent use of product for raw consumption (e.g., ~~close area to harvest~~ avoid harvesting from a specified lease/harvest area or divert product for further processing).
 - Where possible, sink-cultivate bivalve molluscs below the thermocline where the growth of pathogenic *Vibrio* spp. should not occur
 - Restrict the time from harvest to refrigeration
 - Relay bivalve molluscs to areas where risk is sufficiently reduced (e.g., relay bivalve molluscs with *V. vulnificus* to high salinity offshore waters)

²³ J. B. McLaughlin, A. DePaola, C. A. Bopp, K. A. Martinek, N. P. Napolilli, C. G. Allison, S. L. Murray, E. C. Thompson, M. M. Bird, and J. P. Middaugh. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. N Engl J Med 14:1463-1470, 2005.

²⁴ FAO and WHO, 2020, the Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood (Microbiological Risk Assessment series, No. 20) (Section 3.6).

3.3 Handling, storage and transport

17. Bivalve molluscs destined to be consumed live or untreated raw should be handled separately from those harvested in other areas destined for post-harvest processing or other treatment to avoid cross-contamination, where stricter parameters are applied to the former.

18. During handling, storage and transport of harvested bivalve molluscs, the following control measures should be applied as necessary, based upon the factors identified in Section 3.1. It is important that any control for *V. parahaemolyticus* and/or *V. vulnificus* is not less than that required for the control of any other pathogenic organisms that may be present in bivalve molluscs.

- Limit time from harvest or first exposure to ambient air temperature to initial refrigeration based on modelling and sampling.
- Minimize time and temperature conditions that would allow the growth of *V. parahaemolyticus* and *V. vulnificus* during wet storage of bivalve molluscs.
- Bivalve molluscs are to be transported at the lowest temperature that minimizes growth of *V. parahaemolyticus* and *V. vulnificus*. The time between refrigeration and reaching a temperature that does not support growth of *V. parahaemolyticus* and *V. vulnificus* should be minimized ~~when the temperature of the bivalve molluscs exceeds the minimum growth temperature for pathogenic vibrios *Vibrio* spp.~~, and the time between harvest and raw consumption should be limited appropriately or the product should undergo additional treatment to reduce pathogenic *Vibrio* levels. Special attention should be paid to maintaining the characteristics of bivalve molluscs to be consumed live following Section 7.3 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003).
- It may be useful to periodically survey levels of *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs at various points in the distribution chain to verify effectiveness of recommended control measures.
- Anyone involved in the harvest, handling, storage or transport of bivalve molluscs should be educated in the relationship between temperature control and growth of *V. parahaemolyticus* and *V. vulnificus* and trained in proper handling, storage and transport.

SECTION IV - ESTABLISHMENT: DESIGN ~~AND~~ OF FACILITIES AND EQUIPMENT

19. Refer to Section ~~IV~~9 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section IV of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

SECTION V - CONTROL OF OPERATION

5.1 ~~Control of Food Hazards~~ Description of products and process

20. Refer to Section ~~5.4~~13.1 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003), the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008) and Section 5.1 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

21. The control measures described in this section generally apply to post-harvest handling and processing. Control of *V. parahaemolyticus* and *V. vulnificus* typically requires the stringent application of Good Hygienic Practices-GHPs and other supportive programs. These prerequisite programs, together with HACCP, can provide a sound framework for the control of *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs.

22. Any control measures or practice selected to significantly reduce or limit but not necessarily completely eliminate *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs (e.g., freezing, high pressure and mild heating), should be adequately validated to ensure that the control measure is effective. ~~They should also be approved by the competent authority.~~ Such validated control measures/practices should be implemented under the HACCP system. *V. parahaemolyticus* is generally more resistant than *V. vulnificus* to any given treatment. Therefore, a process that is effective for *V. vulnificus* may not be as effective for *V. parahaemolyticus*.

5.2 ~~Key aspects of hygiene control systems~~ GHPs

5.2.1 Time and temperature control

23. Refer to Section 4.1 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003). Time and temperature control to reduce the temperature to the point that *V. parahaemolyticus* and *V. vulnificus* do not grow should be used and maintained during processing operation and subsequently until consumption.

5.2.2 Specific process steps

24. Bivalve molluscs destined to be consumed live or untreated raw should be distributed-handled separately from those destined for post-harvest processing or other treatments.

5.2.3. Microbiological ~~cross-contamination~~

25. Control measures should be in place to avoid cross contamination between bivalve molluscs destined to be consumed live or untreated raw and those that have been subject to harvested in other area destined for post-harvest processing or other treatment.

SECTION VI – ESTABLISHMENT: MAINTENANCE AND SANITATION, CLEANING AND DISINFECTION, AND PEST CONTROL

26. Refer to Section ~~VII~~11 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section VI of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

SECTION VII - ESTABLISHMENT: PERSONAL HYGIENE

27. Refer to Section ~~VII~~12 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section VII of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

SECTION VIII – TRANSPORTATION

28. Refer to Section ~~VIII~~15 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and the Section VIII of *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

SECTION IX - PRODUCT INFORMATION AND CONSUMER AWARENESS

29. Refer to Section ~~VIII~~14 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section IX of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

30. In addition, programs for consumer information should be directed at consumers with increased susceptibility to contracting vibriosis (see para. 100 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*) to help consumers make informed choices about purchase, storage, shelf-life labelling and appropriate food preparation, handling and consumption of live and raw bivalve molluscs, taking into consideration the specific regional conditions and consumption habits.

9.3 Product Labelling

31. Refer to Section 9.3 (Product Labelling) of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood* and Section ~~1~~1-7 and ~~2~~2-7 of the *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN/CXS 292-2008).

9.4.1 Consumer education

32. Refer to Section 9.4 (Consumer education) of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

33. Programs for consumer education should inform consumers of safe consumption practice and handling and preparation of bivalve molluscs aimed at avoiding food safety risks associated with *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs.

SECTION X - TRAINING AND COMPETENCE

34. Refer to Section X10 of the *General Principles of Food Hygiene*, (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products*, (CXC 52-2003) and Section X of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

PART II. BIVALVE MOLLUSCS CONSUMED IN PARTIALLY TREATED STATE²⁵

SECTION III - PRIMARY PRODUCTION

3.1 Environmental ~~hygiene~~control

35. Refer to Section ~~3.18.1~~ of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section 3.1 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

36. The controls described in Section III (Primary production) of Part I should be implemented. The combination of measures of the treatment and those described in Section III of this part should achieve at least an equivalent level of protection to the level of protection provided for raw or live bivalve molluscs in Section III of Part I.

37. If data on log reduction achieved by partial treatment is available, predictive tools in Part I could be applicable.

3.2 Hygienic production of ~~food~~ sources

38. Refer to Section ~~3.28.2~~ of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section 3.2 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

- The control measures described in Section III (Primary production) of Part I should be implemented to achieve at least an equivalent level of protection for bivalve molluscs to be consumed live or raw ~~despite the fact that even though~~ these bivalve molluscs are to be consumed after partial treatment.

3.3 Handling, storage and transport

39. Refer to Section ~~3.38.3~~ of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section 3.3 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

40. The control measures described in Section III (Primary production) of Part I should be implemented to achieve at least an equivalent level of protection for bivalve molluscs to be consumed live or raw ~~despite the fact that even though~~ these bivalve molluscs are to be consumed after partial treatment.

SECTION IV - ESTABLISHMENT: DESIGN ~~AND~~ OF FACILITIES AND EQUIPMENT

41. Refer to Section ~~IV.9~~ of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and the Section IV of the Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood.

SECTION V - CONTROL OF OPERATION

5.1 ~~Control of food hazards~~ Description of products and process

42. Refer to Section ~~5.413.1~~ of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003), the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008) and Section 5.1 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*. Competent authorities should ensure that the food business operator is able to verify the delivery of any partial treatment and additional control measures necessary to ~~assure~~ ensure the safety of the product.

43. The controls described in this section generally apply to post-harvest handling and processing. Control of *V. parahaemolyticus* and *V. vulnificus* will typically require the stringent application of Good Hygienic Practices

²⁵ Part II applies only to products which are partially treated, excluding post-harvest processing. For products in thoroughly treated state, refer to relevant parts of the Good Hygienic Practices as specified in the *General Principles of Food Hygiene* (CXC 1-1969), *Code of Practice for fish and fishery products* (CXC 52-2003) and other applicable Codex documents as those are generally suitable to control *V. parahaemolyticus* and *V. vulnificus* in fully cooked bivalve molluscs.

GHPs and other supportive programs. These prerequisite programs, together with HACCP, can provide a sound framework for the control of *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs.

44. *V. parahaemolyticus* is generally more resistant than *V. vulnificus* to any given treatment. Therefore, a process that is effective for *V. vulnificus* may not be as effective for *V. parahaemolyticus*. Any measure or practice to significantly reduce or limit but not necessarily completely eliminate *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs should be adequately validated to ~~assure~~ensure that the control measures are effective and such validated control measures ~~as practiced~~ should be implemented under an HACCP system.

5.2 Key aspects of hygiene control systems GHPs

5.2.1 Time and temperature control

45. Refer to Section 4.413.2 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003). The partial heat treatment of bivalve molluscs should ensure that the internal temperature of the bivalve molluscs reaches the temperature to ~~ensure~~ secure a reduction of *V. parahaemolyticus* and *V. vulnificus*. Achievement of the validated time and temperature treatment should be ~~ensured~~ guaranteed. After partial heat treatment, growth of *V. parahaemolyticus* and *V. vulnificus* should be controlled.

5.2.2 Specific process steps

46. The partial treatment of bivalve molluscs ~~by means other than heat~~ should be validated to ensure the intended reduction of *V. parahaemolyticus* and *V. vulnificus*. The parameters (e.g., target pH, salt concentration, water activity) should be controlled, monitored and verified.

5.2.3 Microbiological ~~cross~~ contamination

47. Control measures should be in place to avoid cross contamination between bivalve molluscs before partial treatment and after partial treatment.]

SECTION VI – ESTABLISHMENT MAINTENANCE AND SANITATION, CLEANING AND DISINFECTION, AND PEST CONTROL

48. Refer to Section 9.411 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section VI of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

~~SECTION VII - ESTABLISHMENT:~~ PERSONAL HYGIENE

49. Refer to Section ~~VII~~12 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section VII of *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

SECTION VIII – TRANSPORTATION

50. Refer to Section ~~VIII~~14 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section 9.1 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

SECTION IX - PRODUCT INFORMATION AND CONSUMER AWARENESS

51. Refer to Section ~~VIII~~14 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section 9.1 of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

9.1 Product Labelling

52. Refer to the *General Standard for the Labelling of Prepackaged Foods* (CODEX STANCXS 1-1985) and Section ~~4~~2-7 Labelling in the *Standard for Live and Raw Bivalve Molluscs* (CODEX STANCXS 292-2008). Where appropriate, product labels should include information on safe handling practices and storage recommendations.

53. In addition, where appropriate, labelling for bivalve molluscs should include advice on specific safe handling practices (e.g., time, temperature) and consumption.

9.2 Consumer education

54. Refer to Section 9.4 (Consumer education) of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.

55. Programs for consumer education should inform consumers of safe consumption practice and handling and preparation of bivalve molluscs aimed at avoiding food safety risk associated with *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs.

SECTION X - TRAINING AND COMPETENCE

56. Refer to Section ~~X~~10 of the *General Principles of Food Hygiene* (CXC 1-1969), Section 7 of the *Code of Practice for Fish and Fishery Products* (CXC 52-2003) and Section X of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*.