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Bivalve depuration: fundamental and practical aspects

Cover photographs:
Clockwise from top left: Horizontal bivalve depuration plant in Goro, Italy (courtesy of Acqua&Co S.r.l.); depuration system in La Rochelle, France, operating vertical bins and horizontal tanks including a protein skimmer unit (courtesy of Acqua&Co S.r.l); bivalves on display at a fish shop in Rome, Italy (FAO/A. Lovatelli); post-depuration bivalve sorting and packing operations taking place in a separate area of a depuration plant in Ferrara, Italy (courtesy of M.GI.B. S.r.l.).

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World bivalve production and consumption has increased significantly during the recent years, going from a combined total for wild catch and aquaculture of approximately 10.7 million tonnes in 1999 to 14 million tonnes in 2006 (FAO fishery statistics). Likewise, the development of freight by air and sea and preservation techniques have enabled consumers, in different parts of the world, to enjoy eating bivalves that were produced in distant waters. Such developments in distribution and trade have in turn led to emerging challenges for consumer protection, particularly in relation to the safety of bivalves from pathogenic micro-organisms. Several species of bivalves are preferably consumed live or raw (e.g. oysters), or lightly cooked (e.g. mussels) which make them a high risk food product category requiring proper control measures to eliminate or reduce to acceptable levels potential biological, chemical and physical hazards. Furthermore, the distribution of frozen raw products also markedly extends the period of time over which contaminated batches may be consumed.

While the best approach to the production of safe shellfish is to grow them in, and/or harvest them from, areas subject to no external sources of pollution, truly unpolluted shellfish growing waters are very rare. Sourcing shellfish from areas with relatively low levels of pollution, followed by the use of depuration, will ensure that the risk of illness from faecal contaminants will be as low as can be practically achieved without thorough cooking. It enables the removal of microbial contaminants from light or moderately contaminated bivalves and thus increases the availability and supply of safe and nutritious bivalves. Furthermore, it enables the industry to meet the legal requirements of many countries which have made depuration of bivalves mandatory under specific circumstances.

However, effective depuration depends on the operation of the systems to a number of recognized principles which are intended to maximize biological activity of the bivalves while enhancing the separation of any excreted contaminants from the seawater in which bivalves are located and to prevent their re-uptake. It is also necessary to operate the centres within which the systems are located according to recognized standards of food hygiene. Without these measures, the operations can actually increase the level of contamination of individual batches or cause cross-contamination from one batch to another. Depuration will also not yield effective or consistent removal of all types of contaminants and operators need to know the limitations of the process.

This document was prepared to provide guidance to the bivalve industry on the construction, operation and monitoring of depuration systems and processes. It is mainly targeted at new operators or those with limited experience, as well as fishery and public health officers who deal with the bivalve industry. This is of particular importance for several developing countries, where the bivalve industry is expanding quickly with the aim of winning an ever larger share of the bivalve international market.

The document is divided into chapters intended to lead the reader from a consideration of the public health problems associated with bivalve molluscs, through the principles of the depuration process to more detailed considerations of the construction and operation of a depuration centre, including the application of the international principles of Hazard Analysis Critical Control Point (HACCP). Finally, there is a short section on checks to undertake in the case of problems being encountered.

This document is part of three FAO technical publications dedicated to bivalve aquaculture. The first volume of this series entitled "*Hatchery culture of bivalves: A practical manual*" (FAO Fisheries Technical Paper No. 471) was published in 2004 and is now available in Arabic, Chinese, English, French and Spanish. The second volume entitled "*Installation and operation of a modular bivalve hatchery*" (FAO Fisheries Technical Paper No. 492) was published in 2006 and is available in English.

The present document was prepared under the overall coordination of Alessandro Lovatelli, Fishery Resources Officer (Aquaculture), Aquaculture Management and Conservation Service (FIMA). The chapter on HACCP was prepared by Lahsen Ababouch, Chief, Fish Utilization and Marketing Service (FIIU).

Abstract

Bivalve molluscan shellfish concentrate contaminants from the water column in which they grow. These contaminants may then cause illness to humans when the bivalves are eaten. For microbial contaminants, the risk is enhanced by the fact that these shellfish are often eaten raw (e.g. oysters) or relatively lightly cooked (e.g. mussels). Limiting the risk of illness depends partly on sourcing the shellfish from areas in which such contaminants are at relatively low levels. The risk may be reduced further by appropriate treatment following harvest.

Depuration (purification) is a process by which shellfish are held in tanks of clean seawater under conditions which maximize the natural filtering activity which results in expulsion of intestinal contents, which enhances separation of the expelled contaminants from the bivalves, and which prevents their recontamination. Depuration was originally developed as one of a number of means to address the problem of a large number of shellfish-associated outbreaks of typhoid (caused by the bacterium *Salmonella typhi*), which caused illness and death in many European countries and in the United States of America at the end of the nineteenth century and beginning of the twentieth century.

Depuration is effective in removing many faecal bacterial contaminants from shellfish. As currently commercially practised, it is less effective at removing viral contaminants such as norovirus and hepatitis A. It is not consistently effective, or is ineffective, in removing other contaminants such as naturally occurring marine vibrios (e.g. *Vibrio parahaemolyticus* and *Vibrio vulnificus*), marine biotoxins (such as those causing paralytic shellfish poisoning PSP, diarrhetic shellfish poisoning DSP and amnesic shellfish poisoning ASP) or heavy metals or organic chemicals.

Effective depuration requires the shellfish to be properly handled during harvest and pre-depuration transport and storage. It also requires proper design and operation of the depuration systems to meet the requirements identified above for removal and separation of contaminants. Likewise the establishments in which the system or systems are located need to be operated to good levels of food hygiene in order to prevent cross-contamination between, or recontamination of, different batches of shellfish.

This document is intended to provide a basic introduction to the public health problems that can be associated with shellfish consumption and to provide guidance as to how a depuration centre, and the associated systems, should be planned and operated. It also includes guidance on the application of Hazard Analysis Critical Control Point (HACCP) plans and associated monitoring. The document is intended to be of use to members of the shellfish industry with no or limited experience in the area and to fishery and public heath officials who may be involved in providing advice to the industry. Supplementary material may be found in the publications given in the bibliography.

Keywords: marine aquaculture, bivalve depuration, pathogenic micro-organisms, faecal contamination, food hygiene, oysters, clams, scallops

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Acronyms

ABS	Acrylonitrile-Butadiene-Styrene
AOAC	Association of Analytical Communities
ASP	amnesic shellfish poisoning
ATCC	American Type Culture Collection
AZP	Azaspiracid
BP	British Pharmacopeia
CAC	Codex Alimentarius Commission
CAC/GL	Codex Alimentarius Commission/Guidelines
CAC/RCP	Codex Alimentarius Commission/Recommended Codes of Practice
CCMAS	Codex Committee on Methods of Analysis and Sampling
CCP	Critical Control Points
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CI	Cyclic Imines
CRL	Community Reference Laboratory
CRM	Certified Reference Material
DA	Domoic Acid
DAP	Defect Action Plan
DPD	diethyl phenylene diamine
DSP	diarrhetic shellfish poisoning
EC	European Commission
EDTA	Ethylene Diamine Tetraacetic Acid
ETCP	Effluent Toxicity Control Program
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FC	Faecal Coliform
FCV	Feline Calicivirus
GBP	British Pound
GRP	Glass-Reinforced Plastic
HACCP	Hazard Analysis Critical Control Point
HDPE	High Density Polyethylene
HMSO	Her Majesty's Stationery Office (United Kingdom)
IFREMER	Institut français de recherche pour l'exploitation de la mer
INIAP	Instituto Nacional de Investigacao Agroria e das Pescas (Portugal)
INRH	Institut National de Recherche Halieutique (Morocco)
IPIMAR	Instituto de Investigacao das Pescas e do Mar (Portugal)
ISO	International Standards Organization
MMGB	Minerals Modified Glutamate Broth
MPN	Most Probable Number
MTEC	Membrane Thermotolerant *Escherichia coli* Agar
NCTC	National Collection of Type Cultures
NLVs	Norwalk-like viruses
NSP	neurotoxic shellfish poisoning
NSSP	National Shellfish Sanitation Program (USA)
NTU	Nephelometric Turbidity Units
PAHs	polynuclear aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PSP	paralytic shellfish poisoning
PTX	Pectenotoxins
PVC	Poly Vinyl Chloride

RFID	Radio Frequency Identification
RIVO	Institute for Fisheries Research (Netherlands)
RNA	Ribonucleic Acid
SG	Specific Gravity
SRSVs	Small Round Structured Viruses
STX	Saxitoxin
TBGA	Tryptone Bile Glucuronide Agar
TNTC	Too Numerous To Count
USDA	US Department of Agriculture
USFDA	US Food and Drug Administration
UV	Ultraviolet
UVPS	Ultraviolet Power Supply
W	Watt
WHO	World Health Organization of the United Nations
YTX	Yessotoxins

Glossary

Aquaculture	Aquaculture, with respect to this Guide, is the raising of bivalve molluscs from the juvenile state under controlled conditions.
Batch – harvested	Shellfish harvested on the same day and from the same area (if classification is necessary, of the same class).
Batch – depurated	Shellfish that have been depurated through the same cycle of the same depuration system.
Bivalve mollusc	Any marine or freshwater mollusc of the class Pelecypoda (formerly Bivalvia or Lamellibranchia), having a laterally compressed body, a shell consisting of two hinged valves, and gills for respiration. The group includes, among others, clams, cockles, oysters and mussels.
Classification of bivalve mollusc harvesting areas	A system for grading harvesting areas based on levels of bacterial indicator organisms in the surrounding seawater (using faecal coliforms in the US) or the shellfish themselves (using *E. coli* within the EU).
Clean seawater	Seawater from any source where harmful microbiological contamination, substances and/or toxic plankton are not present in such quantities as may affect the health quality of fish, shellfish and their products (Codex Alimentarius Code of Practice).
Coliform	Gram negative, facultatively anaerobic rod-shaped bacteria which ferment lactose to produce acid and gas at 37 °C. Members of this group normally inhabit the intestine of warm-blooded animals but may also be found in the environment (e.g. on plant material and soil).
Control (verb)	To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.
Control (noun)	The state wherein correct procedures are being followed and criteria are being met.
Control measure	Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.
Corrective action	Any action to be taken when the results of monitoring at the critical control point indicate a loss of control.
Culling	The process of separating dead or broken shellfish (and other species) from the live, intact shellfish.
Critical Control Point	A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.
Critical limit	A criterion which separates acceptability from unacceptability.
Deviation	Failure to meet a critical limit.

Depuration cycle The depuration process from the point at which the shellfish are immersed in the seawater and all of the conditions for depuration process are in the correct range until the time when depuration is ended, e.g. by draining the tanks. If conditions go out of range then the cycle must be identified as starting again for the purposes of the depuration period.

Escherichia coli A species of bacterium that is a member of the faecal coliform group (see below). It is more specifically associated with the intestines of warm-blooded animals and birds than other members of the faecal coliform group. Traditionally, *E. coli* produce indole from tryptophan at 44 °C. Now determined on the basis of the possession of β-glucuronidase activity.

Faecal coliforms Coliforms (see above) which can produce their characteristic reactions (e.g. production of acid from lactose) at 44 °C as well as 37 °C. Usually, but not exclusively, associated with the intestines of warm-blooded animals and birds.

Flow diagram A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

Geometric mean The geometric mean of a series of N numbers is the N^{th} root of the product of those numbers. It is more usually calculated by obtaining the mean of the logarithms of the numbers and then taking the antilog of that mean. It is often used to describe the typical values of a skewed data set such as one following a log-normal distribution (see below).

Growing or harvesting area Brackish and marine areas approved for the production or harvesting of bivalve molluscs either by natural growth or by aquaculture destined for human consumption. The growing areas may be approved as production or harvesting areas for bivalve molluscs for direct consumption, or they may be approved as production or harvesting areas for bivalve molluscs for either depuration or relaying (Codex Alimentarius Code of Practice).

HACCP A system which identifies, evaluates, and controls hazards which are significant for food safety.

HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

Hepatitis A virus A 27nm diameter virus that contains RNA as its nucleic acid. It is transmitted by the faecal-oral route and although most infections are inapparent or mild feverish episodes, it can cause inflammation of the liver resulting in jaundice.

Live bivalve molluscs Bivalve molluscs that are alive immediately prior to consumption.

Log-normal distribution	A log-normal distribution is one in which the logarithms of the values have a normal (bell-shaped) distribution. Environmental monitoring data for many bacteria follow a log-normal distribution.
Monitor	The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Norovirus	Noroviruses are small, 27 to 32 nm diameter, structured RNA viruses which have been implicated as the most common cause of nonbacterial gastroenteritis outbreaks. (They were formerly called Small Round Structured Viruses [SRSVs] and Norwalk-like viruses [NLVs]).
Percentile	The *pth* percentile of a series of observations (measurements) is the value such that *p* percent of the observations fall at or below it. Thus the 95th percentile is the value below which 95 percent of the observations fall.
Potable water	Water of sufficient quality as to be safely used for drinking, whether it is used for that or another purpose. It should at least conform to WHO Guidelines (WHO, 2004) and may need to meet requirements in local legislation.
Production area	Any sea, estuarine or lagoon area, containing either natural beds of bivalve molluscs or sites used for the cultivation of bivalve molluscs, and from which live bivalve molluscs are taken.
Relay area	Any sea, estuarine or lagoon area with boundaries clearly marked and indicated by buoys, posts or any other fixed means, and used exclusively for the natural purification of live bivalve molluscs.
Relaying	The removal of bivalve molluscs from microbiologically contaminated growing area to an acceptable growing or holding area under the supervision of the agency having jurisdiction and holding them there for the time necessary for the reduction of contamination to an acceptable level for human consumption (Codex Alimentarius Code of Practice).
Step	A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.
Validation	Obtaining evidence that the elements of the HACCP plan are effective.
Verification	The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

Chapter 1

Introduction

In this manual, the term shellfish will be used widely to describe bivalve molluscan shellfish and, in this context, the term should not be taken to include cephalopods, crustaceans or gastropods.

Depuration (purification) is a technique applied in many parts of the world for the removal of microbial contaminants from light to moderately contaminated bivalve molluscan shellfish by placing them in tanks of clean seawater such that they undertake their normal pumping activity for a period of time that may range from several hours to days (see Part 3 for more details). It is usually undertaken because it is required by regional, national or local legislation but may also be applied at the discretion of the industry to protect their customers, demonstrate due diligence, or to satisfy the requirements of legislation in other regions or counties in order to be able to export to these.

In Europe there is a long history of the use of the process to overcome the problems caused by faecal contamination of shellfish harvesting areas due to the large numbers of people living in coastal locations and to extensive animal husbandry. While there is also a long history of depuration in the United States of America, the wider availability of relatively pristine coastal waters there has allowed greater attention to be placed on harvest of shellfish from such locations rather than removal of contamination post-harvest. Depuration has also been practiced relatively extensively in Australia and Japan, but to a limited extent in New Zealand. In general, shellfish marketed commercially in many other parts of the world have not been subject to specific hygiene requirements and thus depuration has not been practised in these areas.

The purpose of this manual is to give guidance to industry on the construction and operation of depuration systems together with aspects of monitoring the depuration process. The principle factors affecting the effectiveness of depuration are the design of the system itself, quality of the seawater used in it, the way that the system and allied processes are operated and the provision of the right physiological conditions for the shellfish for a sufficient length of time. All of these factors will be examined, and the associated requirements of legislation will be identified for a number of countries around the world. The justification for concentration on the requirements of the European Union (EU) and United States of America is that these two trading blocks tend to drive many of the controls applied in other countries which wish to export shellfish to them.

Although depuration is based on the provision of the correct physiological conditions for the shellfish to undertake their pumping activity, peak effectiveness for microbiological removal, especially of viruses, occurs within a narrower range than that over which the shellfish exhibit such activity. Limits for variables such as temperature and dissolved oxygen given in the literature or stipulated by regulatory bodies may therefore not yield optimum removal of the pathogens. For example, it is known that depuration of viruses from Pacific oysters (*Crassostrea gigas*) is much more effective at 18 °C than at 8 °C in northern temperate countries.

Depuration will only remove light to moderate levels of microbial contaminants and cannot be used for heavily contaminated shellfish. There are also limitations as to the types of microbes that can be successfully removed by the process and these limitations will be emphasized.

In general, the best approach to the production of safe shellfish is to grow them in, and/or harvest them from, areas where the water is not subject to faecal contamination (Approved areas under the US system and class A areas under the EU system; see Section 2.3). The use of depuration in addition to harvesting from clean areas will ensure that the risk of illness from contaminants of faecal origin will be as low as can be practically achieved without thorough cooking.

Other considerations that need to be taken into account with regard to production of safe shellfish are the presence of naturally occurring pathogenic vibrios, phytoplankton-associated biotoxins and chemical contaminants such as heavy metals and organic chemicals. These latter contaminants will be considered briefly in Section 3.

Some general information on the extent and nature of depuration undertaken in a number of countries is summarized in Table 1.1.

Table 1.1: Depuration in selected countries (as of December 2006)

Country	Estimated Number of approved plants	Main species depurated	Types of systems	Types of seawater disinfection
China	7	Clams and oysters	Recirculating; flow-through	UV; ozone
France	1422	*Crassostrea gigas*; *Mytilus edulis*; *Mytilus galloprovincialis*; *Ostrea edulis*; *Cerastoderma edule*; *Ruditapes decussatus*; *Tapes philippinarum*	Static; recirculating; flow-through	UV; ozone; chlorine; aeration
Ireland	20	*Crassostrea gigas*; *Mytilus edulis*; *Ostrea edulis*	Recirculating	UV; borehole water
Italy	114	*Tapes philippinarum*; *Mytilus galloprovincialis*; *Chamelea gallina*	Recirculating; flow-through	UV; ozone; chlorine
Malaysia	2	*Crassostrea iredalei*; *Crassostrea belcheri*	Recirculating	UV
Morocco	2	*Crassostrea gigas*; *Ruditapes decussatus*; *Mytilus galloprovincialis*; *Perna perna*	Static; recirculating	UV; chlorine
Netherlands	10	*Mytilus edulis*; *Crassostrea gigas*; *Ostrea edulis*	Recirculating; flow-through	UV or not disinfected
Philippines	1	*Crassostrea iredalei*; *Perna viridis*	Static; flow-through?	UV; ozone; chlorine; PVP-iodine
Portugal	22	*Ruditapes decussatus*; *Ostrea* spp; *Crassostrea angulata*; *Mytilus* spp.	Static; recirculating; flow-through	UV; chlorine
UK	82	*Mytilus* spp.; *Crassostrea gigas; Ostrea edulis*; *Tapes philippinarum*; *Ruditapes decussatus*; *Cerastoderma edule*	Recirculating; flow-through	UV
Japan	>1000	Oysters and scallops	Static; recirculating; flow-through	UV; ozone; chlorine; electrolysation
Spain - Galicia	60	Mussels; clams; cockles; oysters	Recirculating; flow-through	Chlorine

This manual is primarily intended to provide information to current or prospective members of the shellfish industry who do not have experience of depuration but are contemplating setting up a depuration plant (Figure 1.1). However, it may also provide additional information to members of the industry whose experience is limited with respect to the variety of systems and practices. It is also intended to provide background information for fishery officers and public health officials who deal with the shellfish industry.

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Figure 1.1: Internal view of two large indoor mechanized bivalve depuration plants in Italy

Chapter 2

Why depurate?

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

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

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

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

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

Table 2.1: Hazards associated with bivalve mollusc consumption

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

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

2.1 BIVALVE MOLLUSC-ASSOCIATED ILLNESS

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

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

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

Table 2.2: Microbial causes of bivalve shellfish-associated illness

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

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

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

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

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

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

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

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

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

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

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

2.2 WHICH SPECIES NEED DEPURATION?

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

2.3 LEGISLATIVE REQUIREMENTS

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

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

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

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

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

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

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

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

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

Table 2.3: EU shellfish harvesting area classification criteria

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4 BIOSECURITY

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

Chapter 3

General principles of depuration

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

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

3.1 RESUMPTION OF FILTRATION ACTIVITY

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

Salinity

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

Table 3.1: Recommended or specified minimum salinity limits

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

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

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

[3] Horoshima Prefecture Regulations.

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

Temperature

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

Dissolved oxygen

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

Table 3.2 Recommended or specified temperature limits for depuration

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

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

[2] Seafish Industry Authority recommendation.

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

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

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

3.2 REMOVAL OF CONTAMINANTS

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

3.3 AVOIDANCE OF RECONTAMINATION

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

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

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

An adequate flow of water within the system is necessary to ensure that depurated faeces and pseudofaeces are taken away from the shellfish. However, especially in

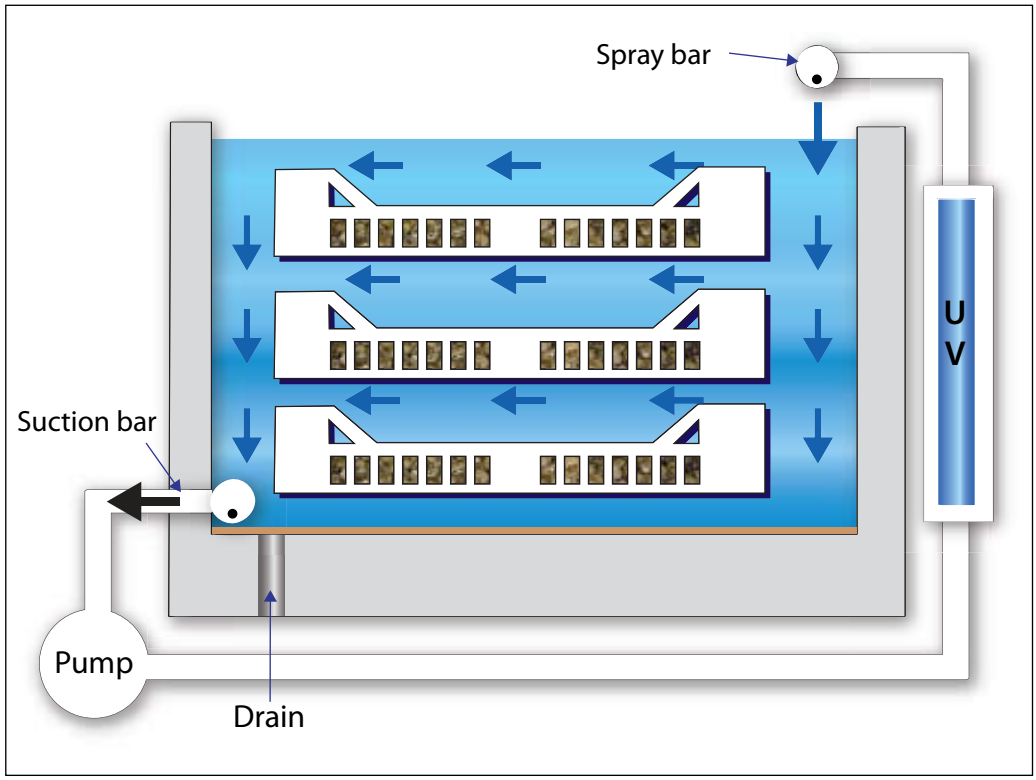

Figure 3.1: Diagram of seawater flow through a loaded tank in a recirculation system

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

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

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

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

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

3.4 MAINTENANCE OF VIABILITY AND QUALITY

Viability and quality is maintained by the following:

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

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

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

3.5 LIMITATIONS OF DEPURATION

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

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

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

3.6 BIOTOXINS

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

3.7 CHEMICAL CONTAMINANTS

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

Chapter 4

Site requirements

4.1 GENERAL LOCATION

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

Planning regulations

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

Access to raw product

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

Access to seawater

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

Access to transport routes for finished product

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

Waste disposal facilities

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

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

4.2 SEAWATER QUALITY

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

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

4.2.1 Natural seawater

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

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

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

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

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

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

4.2.2 Artificial seawater

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

4.2.3 Saline borehole water

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

4.3 ACCESS TO UTILITIES AND LABOUR

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

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

Chapter 5

Plant design and construction

5.1 GENERAL PLANT CONSIDERATIONS

Plants should be constructed in such a way as to prevent stored raw material, the depuration systems, depurated and packaged product, and associated processes, from contamination from airborne or pest-borne contamination and should not be subject to flooding. Preferably, the systems themselves, and associated processes, should be sited within buildings in order to aid control of temperature and contamination. Where this is not possible, systems should be covered during operation and procedures put in place to protect pre- and post-depuration shellfish from contamination and extremes of temperature and exposure to direct sunlight.

Internal surfaces should be made of materials that are easily cleaned and of materials that will not be affected by the use of appropriate disinfectants. In the United States of America, the NSF White Book® Listing has replaced the now terminated US Department of Agriculture (USDA) Listing of Proprietary Substances and Nonfood Compounds. Registered products are listed on the NSF Web site (www.nsf.org/usda/psnclistings.asp).

Floors should be made of easily cleanable material and should slope towards drainage points. Windows and doors should be constructed so as to prevent access of birds and animals.

The product flow should be from dirty to clean going through the steps below in sequence:

1. Receipt of harvested product (through its own door)
2. Indoor pre-depuration storage
3. Washing, debyssing (for mussels) and culling
4. Into depuration tank
5. Depuration
6. Out of depuration tank
7. Washing (may be undertaken in tank as long as the shellfish are not reimmersed)
8. Culling
9. Grading (if necessary) and packing
10. Dispatch of finished product

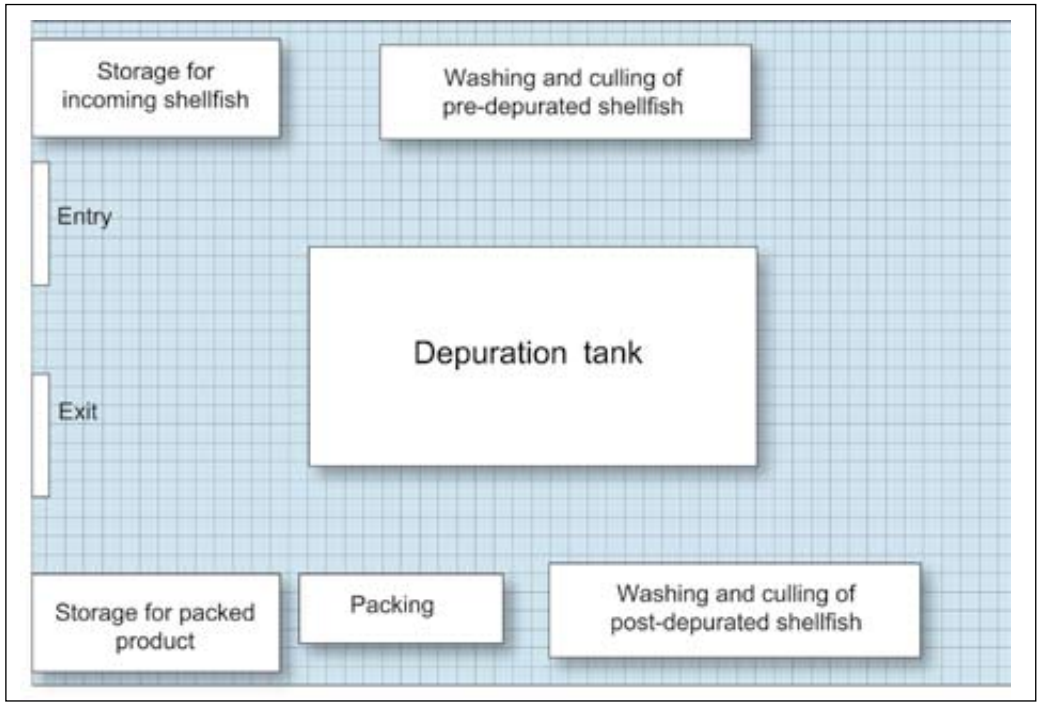

Figure 5.1: Example of a layout of a small-scale depuration facility

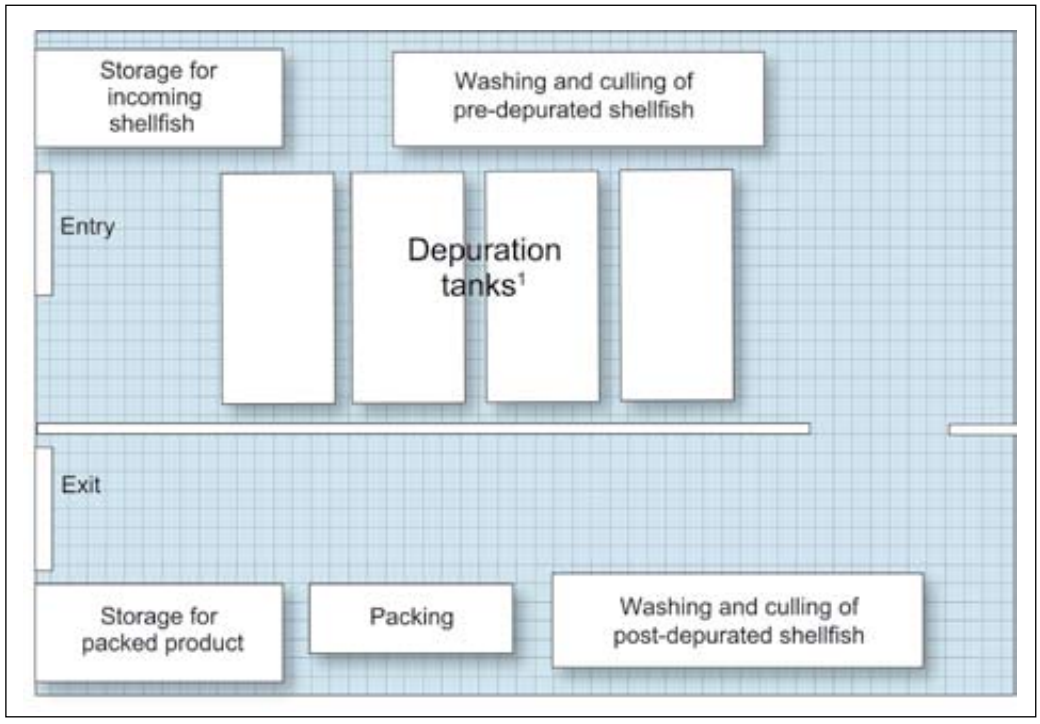

Figure 5.2: Example of a layout of a large-scale depuration facility

[1] Four tanks are shown for the purposes of illustration only: there may be many more in practice. The multiple tanks may be part of separate or multiple systems (depending on whether their seawater supply is common).

Product flow charts from harvest to distribution are given in Appendix 1.

It is strongly recommended that the area used for grading and packing of washed and culled depurated product be physically separated from the rest of the plant by a wall and doorway.

QIAO QING-LIN

Figure 5.3: Interior of a large depuration plant in China

Areas for staff, such as rest rooms and toilets, together with office space, should also be physically separated from the processing area.

While adequate lighting is necessary for the health and safety of staff, the lighting in the vicinity of the tanks themselves should be subdued during the depuration cycle as the animals will not function properly in bright light conditions.

Figure 5.1 shows a schematic layout of a small-scale plant and Figure 5.2 shows a schematic layout of a larger-scale plant. The figures do not show any ancillary facilities such as office space and staff changing rooms which should be located in separate rooms to the product processing area(s).

Figure 5.3 shows part of the interior of a large depuration facility in China.

5.2 DEPURATION TANK DESIGN AND CONSTRUCTION

Tanks, connecting pipework and internal fittings should all be constructed of materials that, under local regulations, are permitted to come directly into contact with foods. Ordinary iron and steel cannot be used due to rapid corrosion, all metal components coming into contact with the circulating seawater should be made of marine grade steel. Other metals should be avoided as some, for example copper, are toxic to the animals.

The tanks themselves are usually made of marine-grade steel, glass-reinforced plastic (GRP) or high density polyethylene (HDPE). If concrete tanks are used, they should be sealed with epoxy resin.

There are a wide variety of tanks and systems. A system is regarded as one or more tanks supplied by a common seawater supply. In general, tanks should be no more than 3 times as long as they are wide so that an even flow of water can be maintained without the presence of any dead-spots. Also, the tank base should have a slope of 1:100 or greater towards the main drain point to assist the washing out of silt and depurated

Table 5.1: Capacities and flow rates for the standard design depuration systems

System	Water capacity (litres)	Maximum capacity for mussels (kg)[1]	Minimum flow rate (litres/min)
Small-scale shallow tank	550	90	20
Medium-scale multi layer	2 600	750[2]	210
Large-scale multi layer	9 200	1 500[2]	160
Vertical stack	650	240	15
Bulk bin system (per bin)	1 100	300[3]	18

[1] The maximum capacity for other species will be less.

[2] The capacity of the medium and large scale systems depends on which type of approved trays are used.

[3] The bulk bin system has only been properly verified for use with mussels.

material after draining at the end of the depuration cycle. It is preferable to have a large bore drain in the tank for final flushing after the depuration cycle that is separate to the normal outlet used during depuration and draining down.

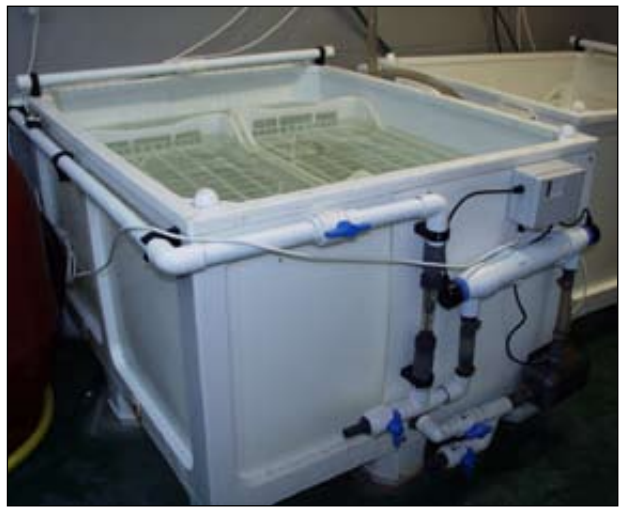

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Figure 5.4: The standard design small-scale shallow tank system

Traditionally, shallow tanks have been used for depuration, with trays stacked at most two high. However, the use of deeper tanks, with higher stacks, increases the throughput of the system without increasing the amount of floor space required. The Seafish Industry Authority (Seafish) in the UK developed and verified a range of standard systems and these cover a range of situations. The systems are summarized in Table 5.1.

Operating manuals are available for all of these standard systems and details of these are given in the Bibliography. Seafish have also produced a general operating manual for non-standard systems from the UK perspective. Figure 5.4 shows the small-scale shallow tank system and Figure 5.5 shows the vertical stack system. Commercial suppliers of depuration systems may provide specific information on the use of their systems.

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Figure 5.5: The standard design vertical stack system

5.3 TRAYS/BASKETS FOR DEPURATION

In most depuration systems, shellfish are placed in trays or baskets prior to depuration. This eases handling and ensures

that the layers of shellfish are not so great that the ones at the bottom cannot open and properly filter seawater. The trays are best made of a suitable plastic such as HDPE and should have sufficient holes or slots as not to provide a barrier to the free flow of water through the shellfish. There should also be holes or slots in the bottom so that egested faeces and pseudofaeces can fall through. Suitable trays are shown in Figure 5.6. The size of tray used will obviously depend on the design and loading arrangement of the tank. Figure 5.6 shows a single tray loaded with clams (*Ruditapes decussatus*).

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Figure 5.6: Example of trays suitable for use in a depuration tank

The baskets/trays should be kept at least 2.5 cm off the base of the tank by battens or other supports in order to allow space for egested faeces and other detritus to settle. The supports should run parallel to the direction of flow so that they do not impede it.

It is not advised that shellfish be put in bags or sacks for depuration for the following reasons:

- If it is intended that the shellfish remain in the same bags in which they are received from the harvesting area then it will not be possible to ensure adequate rinsing, sorting and removal of dead shellfish, other species and general detritus prior to their being placed in the tanks.
- Shellfish that are packed tightly into bags will not be able to open sufficiently to ensure adequate depuration. It would presumably be possible to specify the density allowed for each type/size of bag but this might be difficult to verify.
- The water flow through the bagged shellfish would be affected by the mesh size of the bag, the density and mass of shellfish. The efficiency of removal and settlement of depurated contaminants would also be affected by these same factors.
- Impairment of the ability of the shellfish to open, and reduction in water flow, removal and settlement of contaminants would all be made worse by placing bagged shellfish in tanks in more than a single layer.
- It would be difficult to control the placing of the bags within the tanks with respect to the water inlet and outlet systems.
- The shellfish would need to be removed from the bags prior to post-depuration rinsing and sorting.

Where trays are stacked one above the other, they should be designed such that there is a space between the shellfish in the upper and lower trays to allow room for the shellfish to increase in volume as they open. For most species 3 cm is adequate but for mussels, 8 cm needs to be allowed. For the same reason, 8 cm of water should be above the top level of shellfish at the start of depuration for mussels and 3 cm for all other

species. It is important that the shellfish are covered by water at all times otherwise they will not depurate.

The bulk bin system developed in the UK allows depuration of mussels in 38 cm deep layers with sufficient aeration being provided by a high flow rate of water downwards through the shellfish. The system has not been verified for other species and there are concerns that animals of other species may not be able to open, and therefore function, properly at the bottom of such a load of other shellfish above. In some countries, deeper systems have been used for mussels but there is no direct evidence that the individuals at the very bottom are able to open and there have also been problems in maintaining sufficient dissolved oxygen within the system.

5.4 PLUMBING AND WATER FLOW ARRANGEMENTS

A single system may consist of several tanks with a common water source (flow-through, recirculation or static). If there is more than one tank then the water should be supplied to all tanks in parallel, rather than sequentially, in order to prevent contaminants from one tank passing to another. The requirements for batch operation applies to the water as well as the shellfish and recirculation systems containing more than one tank connected to the same water supply must be started and stopped at the same time – the shellfish in all of the interconnected tanks form a single batch operation.

The flow of seawater in a flow-through system is shown in Figure 5.7 and in a recirculating system in Figure 5.8.

Plumbing should be made of non-corrosive, food-grade materials. ABS (acrylonitrile-butadiene-styrene) plastic is widely used for this purpose although PVC (polyvinyl chloride) is also suitable. Flow of disinfected water is preferably introduced into the

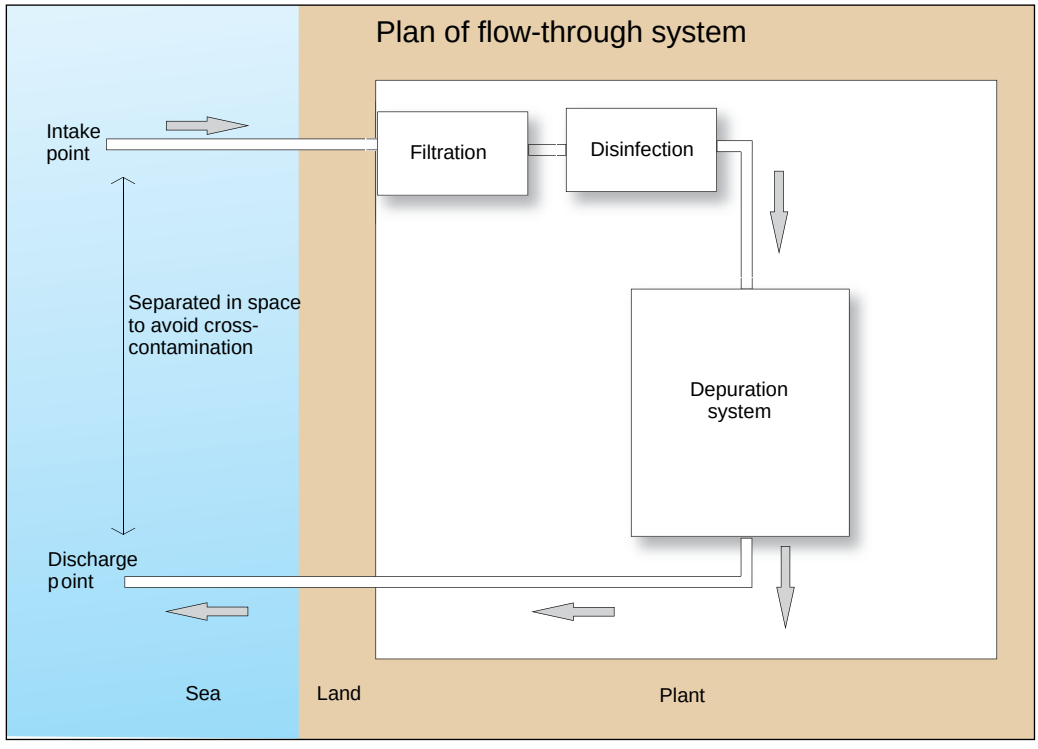

Figure 5.7: Flow of seawater in a flow-through system

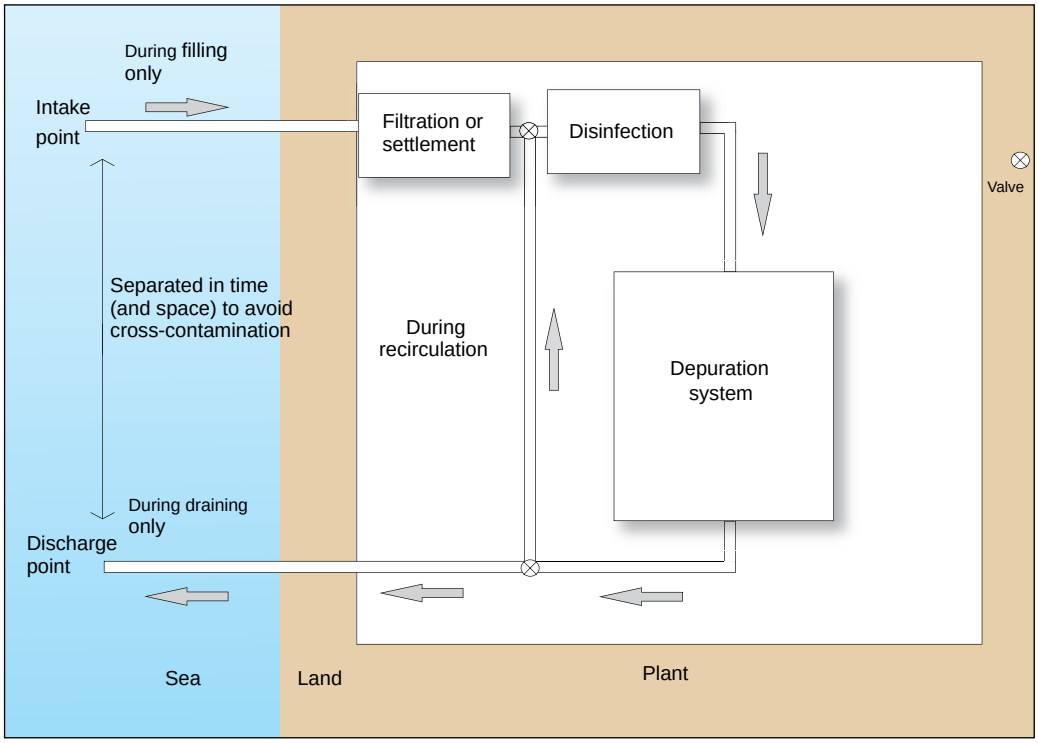

Figure 5.8: Flow of seawater in a recirculating system

tank by means of a spray bar onto the surface of the water at one end, with take off via a suction bar a few centimetres off the base of the other end of the tank (to avoid taking up sedimented material). Both consist of pipes with a series of holes along their length – these provide a relatively even inlet and outlet rates across the width of the tank. Having the inlet and outlets at the top and near (not at) the bottom of the tank respectively also means that the flow should go through as well as across the shellfish. The latter is also maximized by arranging loaded baskets across the width and depth of the tank so that water has to flow through the baskets rather than around them: sufficient space has to be left above the shellfish for the extra depth taken up by opening and moving during depuration and for them to still be totally immersed.

In a recirculation system using UV, the water will then pass through the pump and UV unit back to the spray bar. In a flow-through system, the water from the suction bar will be discharged into the environment or drainage system. An alternative approach to the use of suction bars has been to use one or more central drain pipes, higher than the depth of the water, provided with a number of holes in such positions that vortices are created which give adequate flows through the shellfish. It is necessary to undertake dye-tracing tests for such systems to show that the pipes supply the correct flow arrangement.

Spray bars or other cascade systems will generally provide sufficient aeration to keep the dissolved oxygen content above 5 mg/l provided that the shellfish to water ratio is sufficiently low, the flow rate is correct for the system, and the water temperature is not too high. Problems with low dissolved oxygen levels occur most frequently with mussels. Types of depuration system that do not involve flowing water (static tanks) usually need some form of aeration anyway. If primary or supplementary aeration is to be provided, it should not impinge directly on the shellfish (otherwise they may not function properly) or cause resuspension of sedimented material. In flow-through or recirculation systems, supplementary aeration is best provided in the space between the spray bar end of the tank and a flow screen placed before the first stack of trays. The

Table 5.2: Minimum flow rates specified in the UK for standard design systems[1]

System type	Small-scale 550-600 litres	Medium-scale 2 000–2 500 litres	Large-scale 4 000–4 500 litres	Bulk bin 1 100 litres Bin	Vertical stack 650 litres sump
Minimum flow rate	20 l/min 1.2m^3/hr	208.3 l/min 12.5 m^3/hr	158.3 l/min 9.5 m^3/hr	108.3 l/min 6.5m^3/hr	15 l/min 0.9m^3/hr

[1] Where a higher flow-rate has been applied during the approval process, this may be specified as the minimum by the authorities due to the differences in system performance introduced by minor variations in plumbing arrangements and system operation.

flow screen consists of a vertical sheet of material (plastic or stainless steel, provided with a number of holes in a regular pattern. In the larger UK standard design tanks, flow screens are placed at either end of the tank in order to help provide an even lateral flow of water.

In systems using spray bars or weirs as the primary form of aeration, the dissolved oxygen concentration will depend on the flow rate as well as the design and loading of the system. General advice has been to specify a minimum of one complete change of volume per hour for recirculating systems. Table 5.2 shows the minimum flow-rates specified for the different standard design systems. In the US NSSP, a minimum flow rate of 107 litres per minute per cubic metre of shellfish is recommended. This value is stipulated in New Zealand unless a lower rate has been shown to be effective during the verification procedure. In Hiroshima Prefecture, Japan, the minimum specified flow is 12 litres per 1 000 oysters/minute. In Morocco, a flow rate is not specified but those utilized in the depuration centres is between 30 and 38 m^3/h. In order to ensure that the flow rate is sufficient for optimal activity, and/or to meet the specifications of the authorities, it is therefore necessary to have a means of measuring flow. An in-line flow meter is shown in Figure 5.9.

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Figure 5.9: In-line flow meter used in a depuration system

The internal surfaces of pumps should not contain materials which will be corroded by exposure to seawater or contribute toxic elements or compounds to it (e.g. copper). Recessed impeller pumps are recommended and these should be of sufficient capacity that the required flow can be obtained by reducing the maximum available flow with a diaphragm valve. This means that the required flow can always be achieved. All units should be fitted with a flow meter so that the flow can be measured and adjusted to the required value.

In some parts of the world, systems are based on tanks which utilise a static, rather than flow system. The tanks are filled from a separate source of disinfected water and then left for the period of depuration. Depletion of oxygen is a concern in such systems and primary aeration may be provided. If the depuration period is extensive then the tanks may be drained and refilled at least once during the cycle in order to replenish oxygen (if primary aeration is not provided) and remove initial egested contaminants. Some of these systems contain powerful forced aeration arrangements which impact directly on the shellfish and cause resuspension of sedimented material – these systems therefore do not comply with the general principles of depuration identified in Chapter 3.

Heating or cooling units may be necessary in order to meet the required depuration temperatures, perhaps only for part of the year

depending on local ambient temperatures and the depuration temperature to be met. Heating or cooling may be provided by placing the units coils directly in the tanks (away from the shellfish) or by diverting water away from the tank to a separate heater/cooler unit. The coils, or internal parts of a heater/cooler unit must not contain materials that will readily corrode, or leach into the seawater. A separate pump should be provided for remote units so that the general flow within the depuration tank is maintained. The heater and/or cooler unit should be thermostatically controlled and be able to maintain the tank temperature within the required range at all times. A combined heater/chiller unit is shown in Figure 5.10.

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Figure 5.10: A combined heater/chiller unit suitable for use in conjunction with a small-scale standard design system

It is also possible to control the temperature of depuration by controlling the temperature of the whole building. This may have advantages for control of temperature for several tanks and other parts of the process at the same time.

5.5 DISCHARGE OF USED SEAWATER

The discharge point for used process water should be located away from the intake point so that there is no chance of the contaminated discharged water being recycled. The siting of the intake and discharge points should also take account of tidal flows, etc., in order to reduce the possibility further. With recirculation systems, the intake and discharge operations can also be separated in time. Discharge of used seawater may require licensing by relevant authorities. There may also be local requirements for disinfection of discharged seawater, e.g. to prevent introduction of shellfish pathogens or release of toxin-producing phytoplankton from imported shellfish.

Chapter 6

Water treatment methods

Disinfection of water may not be required if the abstraction point is located within an area classified as of a quality whereby shellfish can be marketed directly for human consumption (EU Class A; US Approved) and the system is of a flow-through design. However, in such circumstances, treatment will provide an extra safeguard against intermittent contamination – it will also provide protection against contamination with pathogens that may be naturally present in the seawater, such as vibrios. If the abstraction point is located within an area of slightly worse water quality, or if the system is of a recirculation design, then disinfection of the source and/or recirculating water will be necessary in order to inactivate pathogens that may be present. Above 5 Nephelometric Turbidity Units (NTU) (approximately 15 mg/l as suspended solids) some attenuation of the UV will occur although the US NSSP gives a turbidity limit of 20 NTU. Care must be taken to ensure that the UV system operates effectively and that particulate material does not accumulate in other parts of the system such as flow meters. Table 6.1 compares the relative advantages and disadvantages of the 3 main methods of disinfection.

Table 6.1: Comparison of three water disinfection systems

Operation/condition	Ultraviolet light	Chlorine/chlorine compound	Ozone
Capital costs	Low	Medium	High
Operating costs	Lowest	Low	High
Installation	Simple	Complex	Complex
Ease of maintenance	Easy	Moderate	Difficult
Cost of maintenance	Low	Medium	High
Performance	Excellent	Possible growth	Unreliable
Source water clarity	High	Low	Medium
Virucidal effect	Good	Poor	Good
Personnel hazards	Medium (eyes, skin)	High	Medium (oxidant)
Toxic chemical	No	Yes	Yes
Residual effect	No	Yes	Some
Effect on water	None	Trialomethanes	Toxic by-products
Operating problems	Low	Medium	High
Contact time (mm)	1–5 sec	30–60 mm	10–20 mm
Effect on shellfish	None	Irritant	Oxidant

Source: Zinnbauer, *Pharmaceutical Engineering* March-April, 1985.

Additional treatment may also be applied to seawater that is recirculated (and especially re-used) in order to reduce concentrations of metabolic by-products from the shellfish (such as proteins and ammonia). These include protein skimmers and biofilters. Where used, these should be operated and maintained strictly according to manufacturer's instructions or technical recommendations. As with all treatment systems, they need to be of sufficient capacity for the volume and flow of water to be treated. Biofilters should be placed prior to disinfection processes. This will ensure that residual chemical disinfectants do not inactivate the micro-organisms on the biofilters and that any micro-organisms released from the filter(s) (which could potentially include pathogens, e.g. vibrios) will be inactivated before reaching the shellfish. Location of skimmers prior to disinfection will also reduce the interference of the by-products with disinfection processes.

It is therefore necessary to place multiple components of water treatment systems in a logical order in order to maximise the performance of each, and the whole system. The target performance of each component should be known (e.g. the target dose for disinfection processes) and each unit should be operated and maintained according to the manufacturer's instructions.

6.1 SETTLEMENT AND FILTRATION

These are the two traditional approaches to reduction in turbidity of source water for depuration.

Settlement

Settlement is most suited to recirculation systems as large storage volumes would be needed for flow-through use. It is undertaken in large tanks for periods of up to a day (usually 12 hours or more) so that large and moderate size particles fall to the bottom of the tank. It is important that the seawater is not disturbed during this period or resuspension will occur. Very fine particles will not settle and therefore the process may not be fully effective in all areas. After settlement, the water to fill a depuration system should be taken from a stopcock situated at least several centimetres above the bottom of the tank in order not to disturb the settled material. For the same reason, the flow rate should be kept relatively low. The settlement tanks should be sited prior to the recirculation unit and the recycled water should not return to the settlement tank. There should be an additional drain point at the base of the tank so that it can be completely drained and cleaned on a regular basis. If settled water is to be kept for more than a day before use, it should be pumped on a short-circuit arrangement, preferably via a UV lamp, in order to keep it from going stale. If this is done, the take off and return points, and flow-rate, should be such as to avoid resuspension of sedimented material. Figure 6.1 shows a diagram of a simple settlement tank.

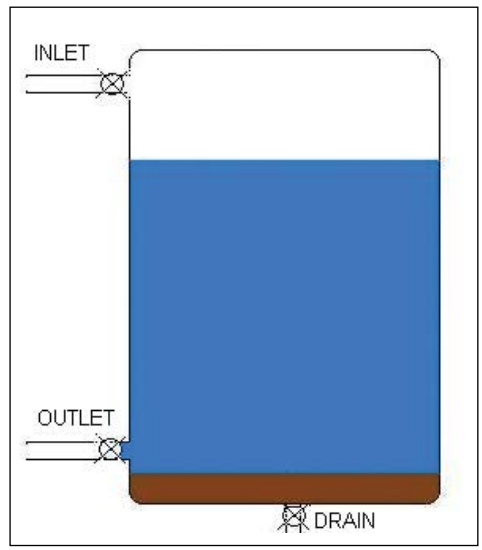

Figure 6.1: Settlement tank used for clarification of seawater

Filtration

Filtration may be used for either flow-through or recirculation systems although its use for the former will depend on the

maximum flow capacity of the filter unit. Filters are used prior to the disinfection process. For recirculation units, the filter should be on the initial fill side of the plumbing system and not within the recirculation system itself as otherwise bacteria and other micro-organisms may grow on the filtration material and form a potential source of contamination within the system.

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Figure 6.2: Pressurized sand filter used in a depuration system

Traditionally, sand filtration units have been used. These are effective in removing particles down to a relatively small size but need to be carefully constructed and maintained in order to be effective. They also have a relatively low maximum flow capacity. Units should either be obtained from a commercial source or built to published specifications. Cleaning and maintenance instructions given by the manufacturer or designer should be strictly followed. A pressurized sand filter included in a depuration system based on UV disinfection is shown in Figure 6.2.

Other filtration units may also be effective for the purpose, including ones with replaceable cartridges or easily cleaned units. It is important that cleanable units are made of materials that will not support the growth of micro-organisms. Again, the manufacturers instructions on cleaning and maintenance (including the replacement of any cartridges, if relevant) be strictly followed.

In Malaysia, filtered seawater is used for depuration without other treatment. The seawater is filtered down to 1 µm to eliminate suspended particles as well as other living flora and fauna in the water (Aileen Tan Shau-Hwai, personal communication). From a microbiological perspective, this process will eliminate bacteria and particle-associated, but not free, viruses.

6.2 ULTRAVIOLET LIGHT

Ultraviolet light (UV) treatment of seawater may be used for both flow through and recirculating systems. Low-pressure lamps have been applied most commonly in depuration systems and the main output of such lamps should be in the UVc region (200 to 280 nm; peak microbiocidal wavelength 254 nm) for disinfection purposes. A single lamp unit consists of a tube in which the UV lamp is contained within a quartz sleeve with the seawater passing down the space between the tube and the sleeve.

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Figure 6.3: UV unit attached to a small-scale shallow tank system

Key: SB = Switch box (on this unit it controls the heater/cooler, pump and UV unit)
UV = UV unit
UVPS = UV power supply

Figure 6.3 shows such a unit on the end of a small-scale shallow tank system (the in-line flow meter is also

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Figure 6.4: Two substantial UV units fitted in a large depuration plant

visible to the left of the UV unit). Figure 6.4 shows two large UV units operating in a large depuration plant (also visible at the end of the room is a protein skimmer with ozonator). The unit therefore has a fixed maximum distance for the UV light to travel – the radial distance between the outside of the quartz sleeve and the inside of the outer tube. Before the development of such enclosed tube units, depuration systems using UV were fitted with lamps situated above water running through a shallow trough or over a weir (Kelly-Purdy units). These are not as efficient as the enclosed systems and are not as safe to operate – their use is therefore not recommended.

A minimum dose of 10 mW/cm^2/sec has been identified as being adequate for use in recirculating systems. This equates to one 30W lamp for a system containing 2 200 litres of seawater. The UV unit manufacturer will specify a maximum flow rate that can be used with the unit.

The efficiency of UV output in the target range decreases with use. The makers of UV lamps tend to specify lifetimes that equate to a remaining efficiency of 80 percent of the original. It is the output at the end of the rated life that ought to be used in determining the size of a UV unit needed for a specific system. For example, the GE G55T8/HO 55W lamp has a recommended useful life of 8 000 hours by which time the nominal output will be 44 W. Lamps need to be replaced at that rated life, even though they are still working, in order to ensure that the correct dose is achieved. It is therefore essential that either each lamp is fitted with an automatic logging mechanism to show the time elapsed since the lamp was last changed or that a manual log is kept. It should be noted that rated lives for lamps are usually based on continuous use and that switching on and off will reduce the effective life.

The dose actually applied to the seawater depends on a number of different factors, including the transmissivity (ability for UV to pass through) of the medium (in this case seawater). The transmissivity will also depend on a number of additional factors, including the turbidity of the seawater and the presence of dissolved inorganic salts or organic material. The amount of UV light actually applied to the seawater also depends on the state of cleanliness of the quartz sleeve containing the lamp(s). Build-up of material on the sleeve will markedly reduce the amount of UV light passing through and it is therefore necessary to have a regular cleaning schedule, following the

manufacturer's instructions for cleaning. It should be noted that any materials used in the cleaning process should be approved for use in food production premises and the units should anyway be thoroughly rinsed following the cleaning process.

UV dosage can be quoted as either the applied dose (usually calculated from the output of the lamp - either theoretical or measured) and the transmissivity of the seawater, or as the received dose (actually measured at the wall of the tube containing the lamp). In practice, devices for measuring UV dose vary greatly in their performance and the most practical way to determine the required dose is to base this on the theoretical performance of the lamp and to control the transmissivity of the water as far as possible (e.g. by including settlement/filtration), as necessary.

UV radiation can be harmful to both the eyes and the skin. The use of lamps in sealed opaque units means that staff are not exposed to the radiation. Some units have translucent end-caps that will transmit visible light emitted by the lamp so that it is obvious whether they are working. Otherwise, other evidence that the lamp is on needs to be provided so that it is possible to check functioning at the start of depuration and at regular periods during the cycle. It must be noted that evidence that the lamp is functioning does NOT mean that the output is satisfactory. Monitoring of use and subsequent replacement after the prescribed number of hours is necessary whether the lamp is on or not.

When dismantling and reassembling units during cleaning or lamp replacement, the manufacturer's instructions should be followed closely so that lamps are not damaged and so that water is kept away from the electrical fittings.

6.3 CHLORINE AND CHLORINE CONTAINING COMPOUNDS

Chlorine was one of the earliest means used to disinfect seawater for depuration. When used with seawater of low to moderate sediment and organic loads, it is an effective bactericide. However, there are concerns with its effectiveness against viruses.

Addition of chlorine is usually undertaken by the use of sodium hypochlorite solution, although chlorine-generating compounds and chlorine gas may be used (NB. The latter is hazardous). In Japan, some plants use in-line electrolysis of seawater to generate chlorine.

For the purposes of depuration, 2 to 3 mg/l free chlorine is normally used for a contact time of up to an hour. In Morocco, the competent authority specifies a maximum free chlorine concentration of 3 mg/l and a contact time of at least one hour.

The amount of chlorine solution required may be determined using the following formula:

$$\text{Volume to add (litres)} = \frac{\text{Final concentration required (mg/l) x tank volume (litres)}}{\text{Concentration of stock solution (mg/l)}}$$

e.g. to get a 3 mg/l final concentration with a tank volume of 1 000 litres and a stock solution concentration of 10 percent (100 000 mg/l) free chlorine:

$$\text{Volume to add (litres)} = \frac{3 \times 1\,000}{100\,000}$$

$$= 0.03 \text{ litres}$$

$$= 30 \text{ ml}$$

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Figure 6.5: Electrolyzer with flow meter used for oyster depuration

Before use it is necessary to reduce free chlorine in the water to less than 0.1 mg/l otherwise the shellfish will not show the required activity and depuration will be impaired. This reduction is achieved by the addition of sodium thiosulphate. There are also concerns that by-products formed in contact with organic materials in seawater may be accumulated by the shellfish and may pose potential long-term health risks in humans.

For chlorination of intake water in Japan using an electrolyzer (see Figure 6.5), containing 3.0 to 3.3 percent (30 to 33 ppt) of NaCl is decomposed by passing over the electrode. Usually, 0.2 to 0.3 mg/l chlorine is used for disinfection. This concentration does not show the toxicity for the oysters and but has been shown to inactivate *E. coli*, *V. parahaemolyticus* and Feline Calicivirus (FCV), a norovirus surrogate.

6.4 OZONE

Ozone is very effective at inactivating both bacteria and viruses. It may be purchased as the gas form in cylinders or produced on-site by means of high energy electrical discharge or UV light (peak wavelength at 185 nm rather than the 254 nm used for UV disinfection). The ozone is then introduced into the seawater via a diffuser in order to get good mixing.

Ozone is a relatively expensive form of disinfection and the gas is very toxic. Therefore, strict safety rules need to be observed. Ozone at a concentration not exceeding 0.5 mg/l (in order to minimize bromate production – see below) can be used to treat seawater in batches for periods up to 10 minutes. This is undertaken in a separate tank to that used for depuration and then the residual ozone has to be discharged from the seawater before use so that it does not adversely affect the animals – this is achieved by aeration.

There are two additional concerns with the use of ozone – the first is that bromates are formed when ozone is in contact with seawater and these are regarded as potential cancer forming compounds. The second is that residual levels of ozone may cause the shellfish to reduce or stop activity, thus reducing the effectiveness of the depuration process.

6.5 IODOPHORS

Systems using iodophors have been used in the past in Italy and attempts have been made to market them in other countries. The intention is that, as well as disinfecting the depuration water, low residual levels of the iodophors within the intestinal tract of the shellfish will have a direct microbiocidal effect, including against viruses. However, concerns have been expressed with regard to the extent of activity against viruses. Systems in Italy now predominantly use UV or ozone.

Chapter 7

Pre-depuration considerations

7.1 HARVEST

Harvesting techniques should not result in marked shock to the animals, or visible damage to the shells as these may result in either a lower depuration efficiency or increased mortality, either in the depuration system or post-depuration. In general, hand-picking and raking techniques cause least shock and damage to the animals while mechanical harvesting techniques have the potential to cause the most. However, the significance depends on both the animal species and the particular method. For example, cockles (*Cerastoderma edule*) show a relatively high rate of damage when harvested mechanically.

7.2 TRANSPORT

Transport procedures should protect the shellfish from contamination, extremes of temperature and physical damage or excessive vibration. Protection from contamination means that the shellfish should be raised off the floor of any vehicle, to keep them out of any draining water, and that they be covered. See Section 7.4 regarding ideal storage temperatures.

Some species are unable to form a water-tight seal when they close and this may give additional constraints on transport times. In the UK, a maximum of 6 hours between harvesting and the start of depuration is specified for both cockles (*C. edule*) and razor clams (*Ensis* spp.). In addition, for razor clams, it is required that they be placed in bundles of a maximum of 12 animals, normally secured by an elastic band, in order that they retain integrity and viability.

7.3 GENERAL HANDLING

Handling procedures at all stages should avoid shock to the animals. In particular, bulk handling should be undertaken in such a way as to avoid dropping the animals onto hard surfaces and to avoid crushing or other damage. Although the majority of animals may survive such procedures their ability to depurate and shelf-life will be impaired.

7.4 STORAGE

Shellfish received at the plant should be stored in such a manner as to prevent contamination and to avoid exposure to extremes of temperature (either heat or cold), preferably within a reception area in the plant. They should be raised off the ground and, if not stored inside, they should be covered. Extremes of temperature can reduce or subsequent depuration effectiveness and high temperatures can lead to multiplication of bacteria, particularly vibrios. The target storage range is normally considered to be 2 to 10 °C, although the characteristics of the local species need to be considered when determining the actual range specified. Local regulations may stipulate other ranges for storage and transport.

7.5 WASHING, CULLING AND DEBYSSING

Any mud or other material must be removed from the outside of the shellfish prior to them being placed into containers (trays/baskets) for loading into the depuration tank(s). The shellfish must also be sorted and inspected and any dead or damaged shellfish, other species, seaweed, etc., must be removed. These operations are necessary to minimize the amount of external contaminants entering the tank(s) and to avoid the possibility of dead shellfish and other species decaying in the tanks. The presence of predators (such as starfish) left in amongst the shellfish may cause stress and prevent them from depurating properly. Mechanical devices are available commercially for the removal of broken shellfish and other debris, including a rinsing facility, but this still needs to be supplemented by visual inspection.

The byssal threads on mussel must be removed before they are placed in containers for depuration. There are a number of commercially available devices for performing this operation.

Chapter 8

System operation

8.1 TRAY LOADING

Different species vary in the maximum weight above them under which they are able to open and pump properly. It is therefore important to take this into account when loading trays or baskets. Table 8.1 gives the maximum depths stipulated in the UK for different species.

8.2 TANK LOADING

In general, it is preferable for the tank to be loaded prior to the seawater being introduced. This avoids the operator contaminating the seawater and enables the trays/baskets to be properly arranged without the possibility of the shellfish opening and ingesting disturbed material. The trays/baskets should be arranged in accordance with the design and approval requirements for the system (see Sections 5.2 and 5.3). Overloading systems will result in depletion of oxygen levels and high concentrations of metabolic end-products (such as ammonia) and reduced effectiveness of depuration.

Small tanks can be loaded manually. Larger tanks may be loaded using mechanical means – an example of this is shown in Figure 8.1. The need for the operator to stand in the tank to load (and unload) the shellfish should be avoided in order to avoid the risk of contamination of the system.

Table 8.1: Maximum depths per tray stipulated in the UK for different shellfish species

Latin name	Common name	Maximum depth
Crassostrea gigas	Pacific oysters	Double layer
Ostrea edulis	Flat oysters	Single layer overlapping
Mytilus edulis	Mussels	80 mm
Cerastoderma edule	Cockles	80 mm
Mercenaria mercenaria	Hard clam	80 mm
Tapes decussatus	Native clam	80 mm
Ensis spp.	Razor clams	Bundles of 12

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Figure 8.1: Mechanical system for loading and unloading tanks

If UV disinfection is used, the system should be filled via the UV unit. This means that the required level of initial disinfection of the seawater should be achieved during a single pass through the unit. In some systems, the plumbing arrangements do not allow this to be done. In this case, the correct volume of seawater is introduced to the tank (without shellfish present) and the initial disinfection is achieved by recirculation through the UV system for a minimum of 12 hours in order to ensure that the entire volume of seawater in the tank has passed through the unit. The shellfish are then added. However, filling via the UV unit is to be preferred.

From a regulatory aspect, maximum loadings may be specified to limit the shellfish:water ratio in the system in order to ensure maintenance of adequate dissolved oxygen concentrations and to prevent build-up of excessive amounts of metabolic products such as ammonia. This will usually be a function of the maximum loading per tray and the number of trays. The maximum loadings stipulated in the UK for the standard design systems are given in Table 8.2. In Morocco, the maximum density authorised by the competent authority is 30 kg/m^2.

There is a recommendation in the US NSSP of a tank seawater volume of at least 6 400 litres per cubic metre of shellfish for hard clams (*M. mercenaria*) and eastern oysters (*Crassostrea virginica*) and 4 000 litres per cubic metre of shellfish for soft clams (*M. arenaria*). In New Zealand, the minimum value of 6 400 litres per cubic metre of shellfish is specified for cockles and oysters unless a lower value is determined, and approved, on the basis of depuration process studies at the time of commissioning while the minimum values for other species have to be based on such procedures.

Table 8.2: Maximum loadings stipulated in the UK for the standard design systems

System type	**Mussels** *Mytilus* species and hybrids	**Cockles** *Cerastoderma edule*	**Oysters**[1] *Crassostrea gigas* and *Ostrea edulis*	**Clam** *Tapes philippinarum* and *Tapes decussatus*	**Hard clam** *Mercenaria mercenaria*	**Razor clam** *Ensis* spp.
Small-scale 550–600 litres	90 kg	30 kg	750	56 kg	72 kg	40 kg
Medium-scale[2] 2 000–2 500 litres	750 kg	110 kg	4150	500 kg	650 kg	145 kg
Large-scale[2] 4 000–4 500 litres	1 500 kg	220 kg	12 000	1 000 kg	1 300 kg	290 kg
Bulk bin[3] 1 100 litres Bin	300 kg	-	-	-	-	-
Vertical stack 650 litre sump total 16 trays	240 kg	80 kg	2 000	168 kg	216 kg	105 kg

[1] The loading for oysters is specified in terms of the number of animals.

[2] The capacity of the medium and large scale systems depends on which type of approved trays are used.

[3] The bulk bin system has only been fully verified for use with mussels.

Shellfish that are not fully immersed will not depurate and so, after loading with shellfish and filling with seawater, it should be checked that there is the minimum recommended depth of seawater above the shellfish.

8.3 BATCH OPERATION

Depuration consists of an all in/all out process for each system. No shellfish must be added to, or removed from, a tank or any part of an interconnected system during a cycle. An interconnected system is one where more than one tank shares the same recirculating water supply or the flow-through supply from one tank comes from another). Where the water flow through single tanks in a system can be isolated from each other, drain down can be carried out at different times once the required depuration period has been completed and the tank to be drained has been isolated from the others. If any disturbance to the system or water flow occurs during a cycle, all shellfish must be replaced in the system and the entire cycle restarted.

8.4 CONDITIONS FOR DEPURATION

The conditions for depuration should follow the principles given in Section 3, be in accordance with local legislative requirements and, where appropriate, be agreed with the local control agency following a formal verification process.

In general, for systems based on flow-through or recirculation, at least 1 change in the seawater per hour is recommended. However, the actual value will depend on the system design (including the shellfish:water ratio) and the species being depurated.

8.5 DEPURATION PERIOD

A wide variety of depuration periods are used around the world, from as short as a few hours to as long as several days. It is important to note that the rate of removal of faecal coliforms or *E. coli* is not necessarily directly related to the rate of removal of pathogens. This especially applies to some of the viral pathogens and marine vibrios. Tailoring depuration periods very closely to the bacterial indicator content of individual batches (which may not relate directly to the pathogen content of that batch) and the theoretical or observed depuration rates of those indicators is therefore spurious. There has been some general tendency towards a period of 48 hours and, in a well-designed and operated system, this should ensure the removal of most sewage-derived bacterial pathogens and give approaching two-thirds reduction of viral pathogens such as Norovirus. Extension of depuration time (e.g. to 5 days) should enhance removal of the viral pathogens, given that the temperature and other conditions are satisfactory (e.g. 18 °C for *C. gigas* in northern Europe).

From a regulatory aspect, a minimum of 42 hours is specified in the UK and 44 hours in the US NSSP. In New Zealand, the stipulated minimum period is 48 hours unless the authority recognizes that the end point requirements will be consistently met by a shorter period. Even in such a case, a minimum of 36 hours is specified although it is also recognised that some species may require longer than 48 hours. Shorter periods than these are used in some countries where a minimum period is not specified by the competent authority and where the industry targets the period primarily at the removal of faecal indicator bacteria. For example, depuration periods of 18–24 hours are commonly used in Italy and in some cases the period may be significantly shorter than this.

8.6 DRAIN DOWN

The water in the tank should normally be drained in the same direction as the normal flow in order to avoid re-suspension of settled faecal material. For the same reason, the rate of draining should be approximately the same as the flow rate during operation. If the normal water take off level (e.g. suction bar) is above the lowest level of shellfish, then an auxiliary lower drainage port should be opened when the water is nearly at that level.

8.7 MONITORING

Monitoring of temperature, salinity and flow rate should be undertaken at least three times during each depuration cycle: at the beginning, in the middle and at the end. If any of these parameters are not within the stipulated ranges (defined by, or as agreed with the local control agency or as given in the Hazard Analysis and Critical Control Point (HACCP) plan then it should be adjusted as appropriate and timing of the process restarted from the beginning.

UV monitoring recommendations are given in Section 6.2. For other seawater disinfection procedures, a test kit should be used to ensure that the appropriate level of disinfectant has been achieved at the start of the contact time for each batch of seawater. The contact time should be recorded. Following disinfection, the residual level of disinfectant should again be determined to ensure that it is below the required levels.

It is important that any method used to determine the concentration of disinfectant is suitable for use with seawater as the salts in this can interfere with some chemical reactions. It is also important to make sure that any method used is suitable for use with the range of concentrations to be expected (normal and abnormal).

Free chlorine is usually measured by a colour reaction with N,N-diethyl phenylene diamine (DPD). Total chlorine is usually measured with the same method after release of bound chlorine by the addition of potassium iodide. Accurate determination requires the use of a meter to determine the level of colour produced by the reaction. Approximate values may be determined by the use of a kit where the resulting depth of colour is compared with a chart.

Ozone is usually added automatically to meet a preset redox potential measured using an appropriate meter. However, the concentration actually achieved in the water undergoing disinfection should be determined occasionally using a chemical method while the residual concentration in the seawater used for depuration should be checked regularly. Both checks may be undertaken using a colour reaction. Two methods for this include bleaching of indigo trisulfonate and a methyl substituted form of the DPD reagent used for chlorine analysis. As with chlorine determinations, kits are available for simple visual comparison while large plants with on-site laboratories may use instrumental measurement to get a more accurate result. A photograph of a kit used in a depuration centre for the measurement of residual ozone is shown in Figure 8.2.

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Figure 8.2: Example of a kit for the measurement of ozone

A suggested record form is given at Appendix 3.

Chapter 9

Post-depuration handling

As with pre-depuration handling, this should avoid recontaminating shellfish, undue shock or vibration to the animals or exposure to extremes of temperature.

9.1 UNLOADING

The water in the depuration system should be drained to below the level of the bottom layer of shellfish before any are removed in order to avoid disturbance and reingestion of sedimented material. Depending on the design and size of tank and containers (trays, baskets, etc.), the shellfish may be removed manually or by a mechanical lifting mechanism.

After unloading, the residual seawater should be drained away and any remaining solid material removed or washed out. The inside of the tank should be scrubbed with a cleaning solution suitable for use in food production (this might be subject to local rules): sodium hypochlorite solutions are often used for this purpose. The tank should then be rinsed thoroughly with potable water or clean seawater in order to remove any traces of the cleaning agent. All remaining rinse water should be drained away before the tank is used again. Every few cycles, the plumbing should be flushed through with the cleaning solution and then meticulously flushed with potable water or clean seawater. This prevents build-up of dirt and slime in the pipes.

9.2 WASHING/DEBYSSING

The shellfish must be rinsed with potable water or clean seawater after depuration in order to remove any adhering solid material such as faeces. This operation may be undertaken in the tank after draining or after the shellfish have been unloaded. At no time must any of the shellfish become immersed in the wash water – adequate drainage must be provided.

Mussels that have been provided with the correct physiological conditions during depuration will embyss and the threads will need to be removed before packing by the same process as used prior to depuration. Preferably, a separate item of equipment should be provided, especially for large-throughput plants. For small plants, the same item of equipment may be used pre- and post-depuration providing all shellfish and

Figure 9.1: Sorting and packing table

other material is removed after pre-depuration use and the equipment is thoroughly cleaned.

Figure 9.1 shows a vibrating table with rinse spray used for post-depuration sorting and packing of mussels.

9.3 PACKING

Packing operations should take place in a separate part of the plant to that used for the other operations and preferably physically separated from those areas (Figure 9.2). Materials for packing should be of food-grade, even though, with most species of shellfish sold live, the packaging should not come directly into contact with the edible parts. Packaging materials may be mesh nets, trays with or without covers, or plastic bags. Local or international regulations (for exported product) may dictate the type of packaging used. The packaging should allow any liquid lost from the shellfish during storage to escape so that the shellfish do not sit in a pool of liquid. Oysters are generally packed with their concave shell downwards.

Depending on the throughput of the plant, commercially available packing machines may be used. These may be set for specific amounts (weights) of shellfish for each pack. Where such machines are used they should be cleaned on a regular basis. For some species of shellfish, e.g. oysters, buyers may require the shellfish to be graded (e.g. by size, weight) and such grading will take place prior to packing. Again, where this grading is undertaken by machine, this should be cleaned regularly.

Local or international regulations may also dictate the type of pack label that is acceptable and the details to be included on the labels. The label itself and the printing thereon needs to be waterproof and the label should stay fixed to the pack during subsequent transport and handling procedures. The labelling itself will often include the species of shellfish, date of packing, shelf-life or use-by date and the approval number of the

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Figure 9.2: Post-depuration bivalve sorting and packaging

packing centre. In the EU, the label must indicate the country of origin (for some specific codes are allotted) and the shelf-life or use-by date can be replaced by the phrase "these animals must be alive when sold". To assist cross-referral to records in the depuration centre, it is useful to include a batch number that indicates the cycle/system (and possibly tank) to which the packed product refers. For commercial purposes, the labels may contain the name of the firm or other details. Examples of labels are shown in Figure 9.3.

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Figure 9.3: Labels attached to the packaging of depurated products

9.4 STORAGE

Packed shellfish awaiting transport (or direct sale from the plant) should be kept in a clean area (or cold room) under temperature controlled conditions, normally 2–10 °C depending on the species in question. This area should be separate to the areas of the plant dealing with the processing prior to the packing stage and may be part of, or lead off, the packing area itself.

9.5 TRANSPORT

Transport should not expose the shellfish to contamination, crushing or extreme vibration in order that the quality and viability of the product is maintained. Transport

should be undertaken in vehicles that are lined with easily cleanable materials. The shellfish themselves should be kept off the base of the vehicle so that any liquid lost from the packs drains away from the load. The temperature should be controlled, normally within the range 2–10 °C depending on the species in question. As with pre-depuration storage and transport, local regulations may stipulate other temperature ranges. International trade, or even slow methods of transport for local markets, may result in potentially long periods between packing and arrival at the final destination and this will increase the difficulty in maintaining the optimum temperature during transport.

Chapter 10

Microbiological monitoring

The ultimate measure of success of depuration relates to its ability to remove the microbial contaminants for which it is carried out while keeping the bivalves live and of good quality. Microbiological monitoring therefore provides the basis on which to judge that this has occurred. However, such monitoring is usually based on faecal indicator bacteria and these are removed more easily than many of the pathogens (especially the viruses) (see Section 3.5). Such monitoring does not, therefore, provide a definitive measure of the safety of the depurated product.

10.1 PROCESS VERIFICATION

Physical assessment of a depuration system as satisfactory and ensuring maintenance of factors affecting physiological activity in the right range for the species in question does not always lead to the system providing satisfactory bacterial reductions. Therefore, local regulations may require that the effectiveness of the system be demonstrated in practice before it is used for depuration of product intended for the marketplace. Such requirements differ markedly. It is usually based on the bacteriological testing of samples from the loaded system pre- and post-depuration and determining whether the reduction in the concentration of faecal indicator bacteria (either faecal coliforms or *E. coli*) is satisfactory. In Europe, the requirements vary between countries and in some standard design systems may only require a single satisfactory verification cycle prior to full approval although non-standard designs may require very thorough validation. Under the US NSSP, product from unverified systems is subject to positive release based on end-product criteria for single cycles while verification is achieved by showing that the general performance over 10 consecutive cycles is satisfactory. The NSSP verification criteria are shown in Table 10.1. Plants which have not achieved full verification over 10 cycles, where a new source of shellfish is used, or where failure of the verification criteria has occurred, the shellfish post-depuration must meet the following criteria:

i) Geometric mean (from three samples) of soft clams not to exceed 110 faecal coliforms/100 g and no single sample to exceed 170; or
ii) Geometric mean (from three samples) of other clam species, mussels, or oysters not to exceed 45 faecal coliforms/100 g and no single sample to exceed 100.

Table 10.1: US NSSP criteria for verification of depuration plant performance

	Faecal coliforms per 100 grams	
Species	**Geometric mean**	**90th percentile**
Soft clams *Mya arenaria*	50	130
Hard clams *Mercenaria mercenaria*	20	70
Oysters	20	70
Manila clams *Tapes philippinarum*	20	70
Mussels	20	70

10.2 ONGOING MONITORING

The microbiological monitoring is usually not undertaken as a primary control in itself, or even as routine monitoring of critical points in the process. Rather, it is done to check that the process is producing the required outcome given the other controls and monitoring procedures that are in place. Usually, the microbiological monitoring will include pre- and post-disinfection analysis of the seawater and pre- and post-depuration analysis of shellfish.

Microbiological monitoring should be undertaken at a frequency stipulated by the local control agency or resulting from the outcome of the HACCP study (see Section 11). The frequencies recommended below are those that should be considered in the absence of those requirements. Where there is more than one tank per system, samples should be randomly taken from at least one tank chosen randomly.

An example record form is given at Appendix 3.

10.2.1 Seawater

The seawater entering the depuration tanks should be monitored for faecal indicator organisms on at least a weekly basis. Samples should be taken aseptically and sent to an accredited laboratory for testing for faecal coliforms and/or *E. coli* using a suitable method(s) (e.g. ISO 9308, part 1, 2 or 3). Neither of these faecal indicator bacteria should be detectable in 100 ml of the disinfected seawater.

10.2.2 Shellfish

On a regular basis, pre- and post-depuration shellfish from the same batch should be tested. The pre-depuration test confirms that the microbiological content of the harvested shellfish is that expected from the classification status of the harvesting area, as well as identify the microbiological load to be reduced by the process, while the post-depuration sample indicates whether depuration has been successful. The results of pre-depuration samples will depend on the microbiological status of the harvesting area. Single post-depuration samples should not exceed 230 *E. coli* (300 faecal coliforms) per 100 grams. Local regulations may require lower post-depuration results than this and a properly designed and operated system should be capable of consistently producing levels of ≤80 *E. coli* (100 faecal coliforms) per 100 grams. A suitable method for the laboratory to use is ISO TS 16649-3 – a standard operating procedure based on this method is given in Appendix 7.

In some countries there are additional requirements for depurated shellfish. For example, in Japan, in addition to the *E. coli* standard of 230 per 100 grams, the bacterial count should be no more than 50 000 per gram and the MPN for *V. parahaemolyticus* should be no more than 100 per gram.

Chapter 11

Hazard Analysis Critical Control Point (HACCP)

HACCP is a system which identifies, evaluates and controls hazards which are significant for food safety (CAC, 2003). It is a science-based and systematic tool that assesses hazards and establishes control systems which focus on prevention rather than rely mainly on end product testing. It not only has the advantage of enhancing the safety of the product but, because of the means of documentation and control, it provides a means of demonstrating competence to customers and compliance with legislative requirements to the authorities.

11.1 BASIC PRINCIPLES OF HACCP

The *Codex Alimentarius* Commission has adopted the basic texts on food hygiene, including HACCP, in 1997 and 1999 and the guidelines for the application of HACCP were revised in 2003 (CAC, 2003).

The HACCP system can be applied from production to consumption and it consists of the following seven principles:

Principle 1: Conduct a hazard analysis
Identify the potential hazard(s) associated with each stage of depuration; assess the likelihood of occurrence of the hazard and identify the measures for their control;

Principle 2: Determine Critical Control Points (CCP);
Determine the points, procedures or operational steps that can be controlled to eliminate the hazard(s) or minimize its (their) likelihood of occurrence;

Principle 3: Establish critical limit(s)
Establish critical limit(s) which must be met to ensure that the CCP is under control;

Principle 4: Establish a system to monitor control of the CCP
Establish a system to monitor control of the CCP by scheduled testing or observations;

Principle 5: Establish corrective action(s)
Establish the corrective action(s) which must be taken when monitoring indicates that a particular CCP is not under control;

Principle 6: Establish procedures for verification
Establish procedures for verification which include supplementary tests and procedures to confirm that the HACCP system is working effectively;

Principle 7: Establish records and record keeping
Establish documentation concerning all procedures and records appropriate to these principles and their application.

11.2 APPLICATION OF THE HACCP PRINCIPLES TO SHELLFISH DEPURATION

Prior to the application of HACCP to a depuration unit, that unit should be operating according to the *International Recommended Code of Practice – General Principles of Food Hygiene* (CAC/RCP 1-1969, Rev.4 2004). Annex 1: *HACCP System and Guidelines for its Application* should be consulted for further information to assist with the design of a specific HACCP plan.

Management awareness and commitment is necessary for implementation of an effective HACCP system. The effectiveness will also rely upon management and employees having the appropriate HACCP knowledge and skills.

If the necessary expertise is not available on site for the development and implementation of an effective HACCP plan, expert advice should be obtained from other sources, which may include: trade and industry associations, independent experts and regulatory authorities. HACCP literature and especially depuration HACCP guides can be valuable and may provide a useful tool for businesses in designing and implementing the HACCP plan.

The efficacy of any HACCP system will nevertheless rely on management and employees having the appropriate HACCP knowledge and skills, therefore ongoing training is necessary for all levels of employees and managers, as appropriate.

The application of HACCP principles consists of the following tasks as identified in the logic sequence for the application of HACCP (CAC, 2003).

A HACCP plan is a document that describes how a depuration plant will apply the above seven principles in its particular depuration establishment. The following sequence for the preparation of a specific HACCP plan is recommended by the Codex Alimentarius (Figure 11.1). It is applied hereafter for shellfish depuration considering only process critical control points and assuming that sanitary CCPs (hygiene practices, cleaning and disinfection, etc.) are implemented as per regulatory requirements.

1. Assemble a HACCP team
The HACCP team should have access to all information necessary for their work. The present manual is a good source of information to the HACCP team to identify the hazards and the control measures.

If the necessary knowledge and skills is not available at the depuration establishment, the team can be assisted by local public health officers, independent expert(s), fisheries extension officers and/or fish inspection officers.

For example, a HACCP team of a hypothetical depuration plant can be formed by:

- The Unit's Safety supervisor with a degree/training in food science/food safety, good experience in shellfish depuration and a special training in HACCP application to depuration
- The Unit's Personnel supervisor with a degree/training in food hygiene, experience in seafood industry and a special training in HACCP application to depuration
- The Unit's equipment maintenance
- An advisor on shellfish safety and regulatory requirements

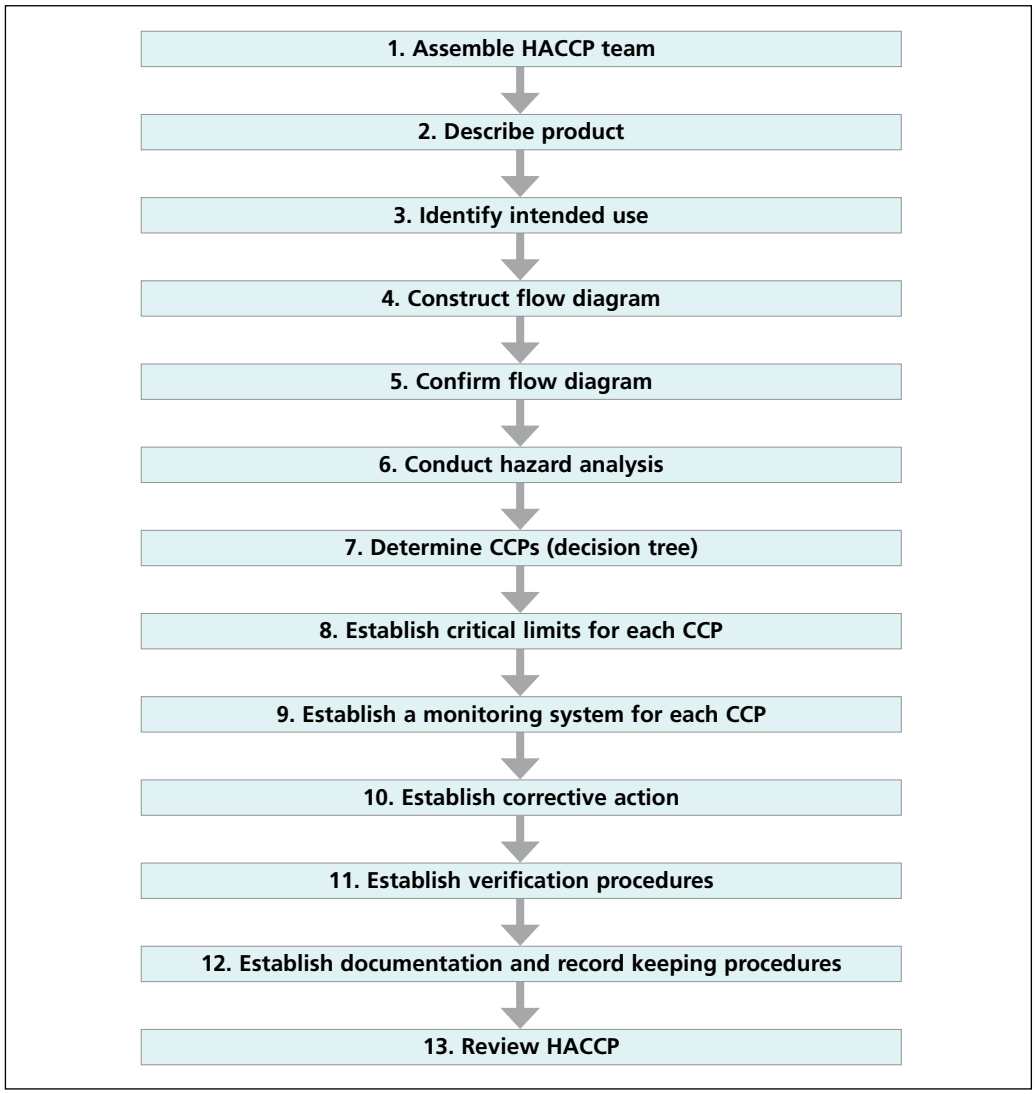

Figure 11.1: Summary of how to implement a HACCP analysis

2. Describe product

A full description of the product should be drawn up, including relevant safety information such as: harvesting area, depuration technique storage conditions, conditions and methods of distribution. The description should at least include the following items:

– Name of the product
– Shellfish species (common and/or scientific name)
– Type of depuration
– Preservation method (live, fresh chilled in ice)
– Packaging method (plastic boxes, polyurethane boxes, other)

An example of product description can be as follows:
"Live oysters (*Crassostrea gigas*) harvested from (locality), depurated for at least 44 hours, using UV disinfected water". The depurated oysters are packed in mesh nets and sold live to retailers and to restaurants.

3. Identify intended use

The intended use should be based on the expected uses by the end user or consumer. It is important to identify if the product is to be used in a way which increases the risk of harm to the consumer, or if the product is particularly used by consumers who are especially susceptible to a hazard. In specific cases, e.g. institutional feeding, vulnerable groups of the population may have to be considered.

For example, a description of the intended use can read as follows: The live oysters (*Crassostrea gigas*) are purchased by restaurants, transported in refrigerated trucks, stored at temperatures of 5 to 10 °C and served live to the customers.

4. Construct flow diagram

A flow diagram should be constructed by the HACCP team (e.g. Figure 11.2). The flow diagram should cover all steps in the operation. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specific operation.

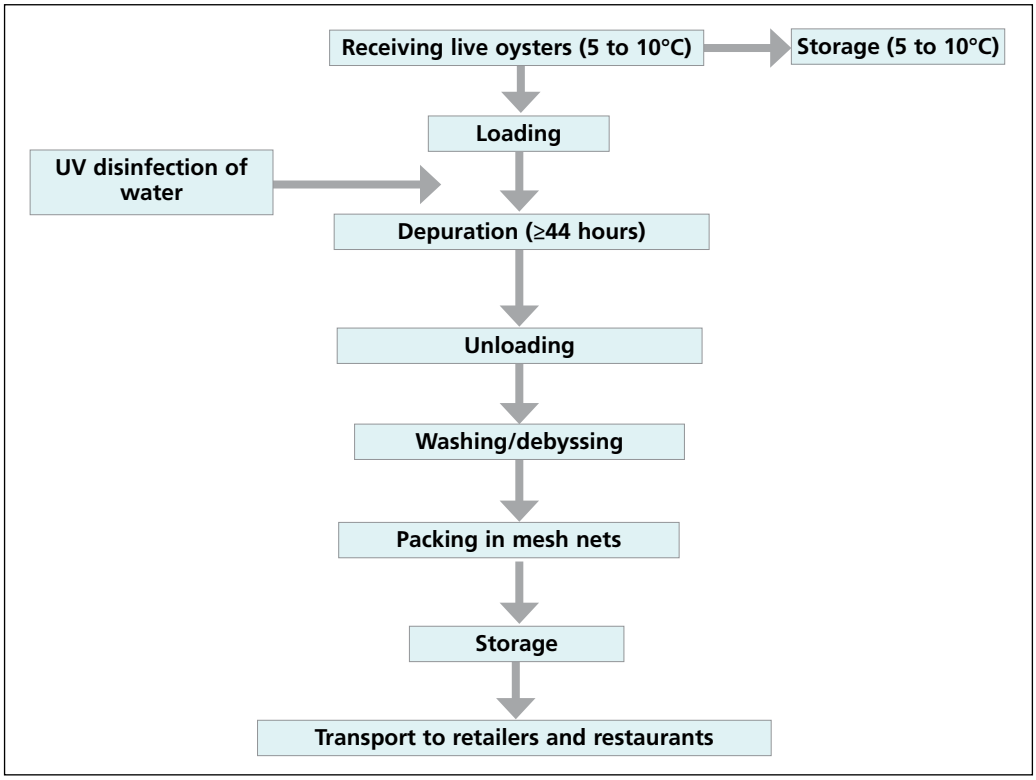

Figure 11.2: Example of a shellfish depuration flow diagram

5. On-site verification of flow diagram

The HACCP team should confirm *in situ* the production operation against the flow diagram during all stages and hours of operation and amend the flow diagram with information such as correct durations, temperatures, etc., where appropriate.

6. List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards (see Principle 1)
The HACCP team should list all hazards that may be reasonably expected to occur during depuration, transportation until the point of shellfish consumption.

A hazard is defined as a biological, chemical or physical agent in, or condition of food, with the potential to cause an adverse health effect.

The HACCP team should next conduct a hazard analysis to identify which hazards are of such a nature that their elimination or reduction to acceptable levels is essential for the production of a safe depurated bivalves.

Hazard analysis is the first HACCP principle and one of the most important tasks for the application of the HACCP system. An inaccurate hazard analysis would inevitably lead to the development of an inadequate HACCP plan.

In conducting the hazard analysis, wherever possible the following should be included:

- the likely occurrence of hazards and severity of their adverse health effects;
- the qualitative and/or quantitative evaluation of the presence of hazards;
- survival or multiplication of microorganism of concern;
- production or persistence in bivalves of toxins, chemicals or physical agents; and
- conditions leading to the above

The HACCP team must then consider what control measures, if any, exist which can be applied for each hazard. More than one control measure may be required to control a specific hazard (s) and more than one hazard may be controlled by a specific control measure.

Consideration needs to be given whether any elements of the process itself will introduce potential hazards. With regard to depuration, these may include disinfectant compounds such as chlorine or ozone used to produce clean seawater and any by-products formed during their use.

Using the information provided in this manual, a hazard analysis for the live oysters delivered to retailers and restaurants, used here as an example (see page 54), is summarized in the HACCP plan (Table 11.1). It includes, among other HACCP information, the hazards identified and the measures selected to control these hazards.

7. Determine Critical Control Points (CCPs)
A CCP is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree (Figure 11.3) recommended by the CODEX which indicates a logic reasoning approach.

There may be more than one CCP at which control is applied to address the same hazard. Likewise, several hazards can be controlled at a single CCP.

The application of the decision tree should be flexible according to the type of operation. Other approaches than the decision tree may be used for the determination of CCPs. If a hazard has been identified at a step where control is necessary for safety, and if no control measure exists at that step or at any other, then the product or the process should be modified at that step, or at an earlier or later stage, to include a control measure.

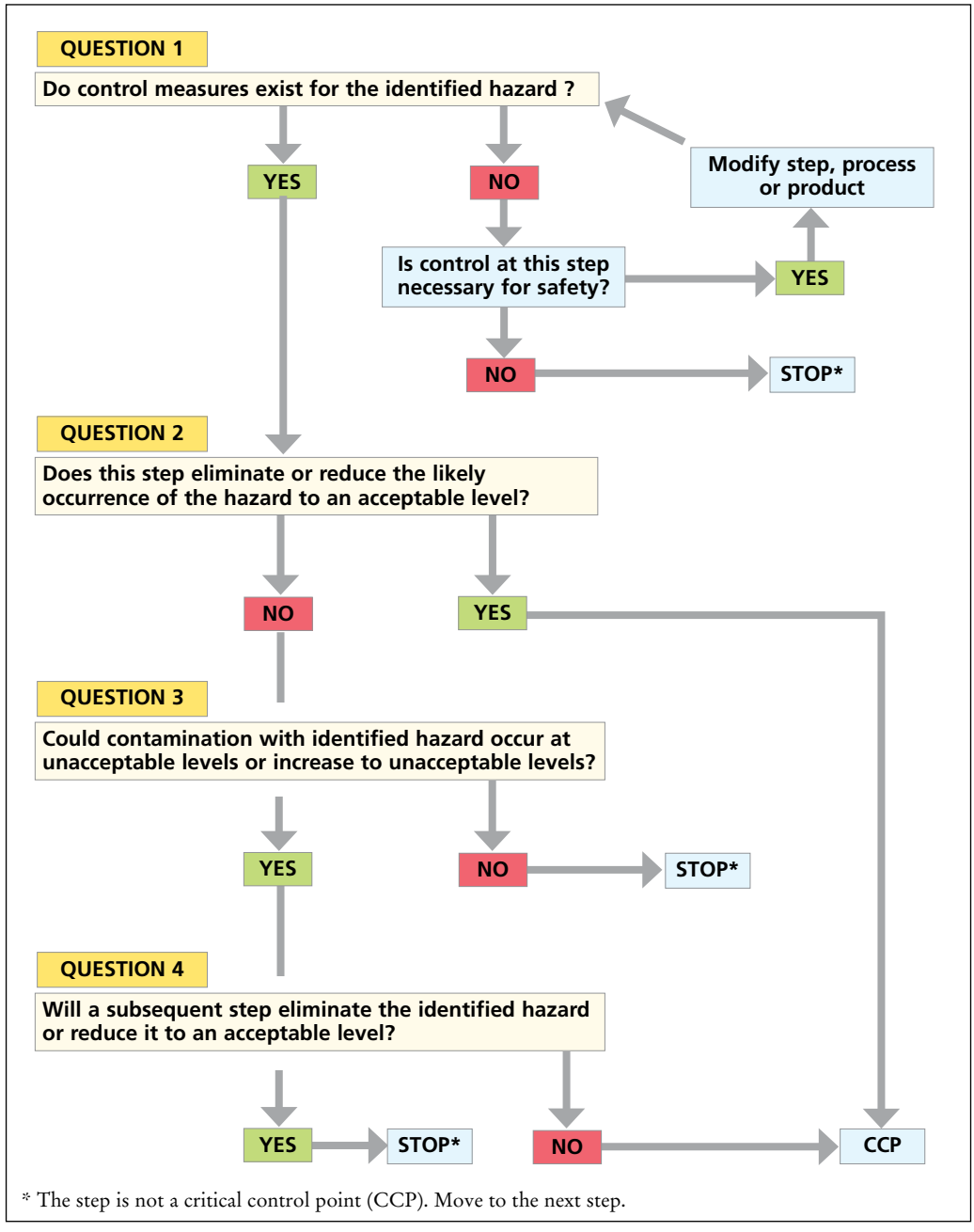

Figure 11.3: Decision tree for the identification of critical control points

As described elsewhere in this manual, depuration as currently commercially practised will not reliably reduce pathogenic marine vibrios, biotoxins or chemical contaminants from potentially hazardous concentrations to those where the product can be considered safe for consumption. CCPs for these hazards must recognise this – they will invariably focus on ensuring that product is received from areas where concentrations in the shellfish are below statutory or recommended safe limits. Current controls on harvesting areas will not ensure that harvested shellfish will be free from pathogenic viruses although the occurrence and concentration will tend to be lower from areas of better water quality, e.g. NSSP approved status or EU class A. Additionally, depuration as currently practised will not ensure removal of viruses but may, if performed according to best practice, reduce the concentration of these. Both of these considerations need to be taken into account when identifying CCPs and applying them within the HACCP plan.

Following is an example of the application of the decision tree to decide whether receiving raw material is CCP for the hazard presence of biotoxins and the hazard presence of salmonella and viruses.

Step 1: Receiving live oysters

Hazard 1: Presence of pathogenic bacteria and viruses

Control measure(s):

1) Purchase live oysters only from a licensed harvester who has harvested them from an approved B area and has tagged the containers or has proper purchase records

Is step 1 a CCP for the considered hazard or not?

Question 1: Do control measures exist for the identified hazard? Yes (measure described above)

Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level? Yes. By applying the control measure 1 described above, we avoid purchase of oysters which can not be rendered safe for human consumption by depuration.

Conclusion: This step is a CCP for the obtention of safe live oysters after depuration

Hazard 2: Presence of biotoxins

Control measure(s):

1) Purchase live oysters only from a licensed harvester who has harvested them from an approved area and has tagged the containers or has proper purchase records

Is step 1 a CCP for the considered hazard of biotoxins or not?

Question 1: Do control measures exist for the identified hazard? Yes (purchase only from licensed suppliers)

Question 2: Does this step eliminate or reduce the likely occurrence of the hazard to an acceptable level? Yes. By using only licensed harvesters that collect only from approved areas we avoid depurating oysters containing biotoxins.

Conclusion: This step is a CCP for the considered hazard

This exercise shall be conducted at each step and for each hazard to identify CCPs. In the present example, the CCP identified using the decision tree are summarized in Table 11.1, along with other useful information.

8. Establish critical limits for each Critical Control Point (CCP)

Critical limits are defined as criteria that separate acceptability from unacceptability. A Critical limit represents the boundaries that are used to judge whether an operation is producing safe products as a result of proper application of the control measures. In other words, critical limits must be met to ensure that a CCP is under control.

Critical limits are set for factors such as temperature, time, chlorine concentration. These parameters, if maintained within boundaries, will confirm that a given hazard is under control at a given CCP.

The critical limits should meet requirements of government regulations and/or company standards and/or be supported by other scientific data. It is essential that the person(s) responsible for establishing critical limits have knowledge of the process and of the legal and commercial standards required for the products.

As an example, the HACCP plan (Table 11.1) defines the critical limits for the measures designed to control the identified hazards at each identified CCP.

9. Establish a monitoring system for each CCP
Monitoring is defined as the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control. The monitoring procedures will determine if the control measures are being implemented and ensure that critical limits are not exceeded. The monitoring procedures must be able to detect loss of control at the CCP.

The purposes of monitoring include the following:

- To measure the performance level of the system's operation at the CCP (trend analysis)
- To determine when the performance level of the system results in a loss of control at the CCP, e.g. when there is deviation from a critical limit
- To establish records that reflect the performance level of the system's operation at the CCP to comply with the HACCP plan

The monitoring procedures should give information on:

What will be monitored (What?)

Monitoring may mean measuring a characteristic of the depuration process or of the product to determine compliance with a critical limit. Monitoring may also mean observing whether a control measure at a CCP is being implemented. Examples include verification of the duration and intensity of a UV treatment.

How critical limits and control measures will be monitored (How?)

Deviation from a critical limit should be detected in as short a time as possible to allow corrective action to limit the amount of adversely affected product. Microbiological testing is rarely effective for monitoring CCPs for this reason. Instead, physical and chemical measurements (e.g. pH, time, temperature, oyster physical appearance) are preferred, as they can be done rapidly and can often be related to the microbiological control of the process. This correlation between rapid measurements and microbiological control needs to be regularly validated.

Equipment used for monitoring procedures should undergo periodic calibration or standardization as necessary to ensure accuracy.

Operators should be trained in proper use of the monitoring equipment and should be provided with a clear description of how the monitoring should be carried out.

Frequency of monitoring (When?)

Where possible, continuous monitoring is preferred; it is possible for many types of physical or chemical methods. Examples of continuous monitoring would include the automatic measurement of free chlorine levels in water.

Where non-continuous monitoring is the chosen system, the frequency of monitoring should be determined from historical knowledge of the process and product. When problems are detected the frequency of monitoring may need to be increased until the cause of the problem is corrected.

Who will monitor (Who?)

Careful consideration should be given to assigning responsibility for monitoring. Once assigned, the individual responsible for monitoring a CCP must:

- Be adequately trained in the CCP monitoring techniques
- Fully understand the importance of CCP monitoring techniques
- Have ready access (be close) to the monitoring activity
- Accurately report each monitoring activity
- Have the authority to take appropriate action as defined in the HACCP plan
- Immediately report critical limit deviation

Examples would include the indication of the Purchase Manager as the responsible for the monitoring procedures at the CCP receiving harvested oysters.

Where to monitor (Where?)

Monitoring takes place at each CCP where a given control measure is applied to control a given hazard.

The HACCP plan (Table 11.1) summarizes the monitoring procedures recommended for the operations described in Figure 11.2.

10. Establish corrective actions

Since the main reason for implementing HACCP is to prevent problems from occurring, corrective actions should be taken when the results of monitoring at the CCP indicate a loss of control. Loss of control can cause a deviation from a critical limit for a CCP. All deviations must be controlled by taking predetermined actions to control the non-compliant product and to correct the cause of non-compliance.

Product control includes proper identification, control and disposition of the affected product. The control and disposition of the affected product and the corrective actions taken must be recorded and filed.

The establishment should have effective procedures in place to identify, isolate (separate), mark clearly and control all products depurated during the deviation period.

Corrective action procedures are necessary to determine the cause of the problem, take action to prevent recurrence and follow up with monitoring and reassessment to ensure that the action taken is effective. Reassessment of the hazard analysis or modification of the HACCP plan may be necessary to eliminate further recurrence.

Examples would include the rejection of oysters not certified as coming from an unauthorized harvesting area or from a non licensed harvester or dealer.

Records should be available to demonstrate the control of products affected by the deviation and the corrective action taken. Adequate records permit verification that the producer has deviations under control and has taken corrective action.

The HACCP plan (Table 11.1) summarizes corrective actions recommended for the operation described in Figure 11.2

For example, the following verification procedure can be recommended for the depuration operation described in Figure 11.2.

Wherever needed but at least weekly, the HACCP team assesses internally all the results of the controls, monitoring and corrective actions and draws conclusions for the subsequent production weeks.

On a longer term, annually, the HACCP team can:

- Evaluate the monitoring and corrective actions data to assess performance and analyses the reason for any loss of control or for complaints from clients and/or control authorities.
- The results of this analysis will be used to update the HACCP manual, identify any internal need for further training and improved practices and performance, maintenance, modify frequency (increase or decrease) of specific monitoring, revise list of approved suppliers.
- An audit by the advisor to assess the performance of each control, monitoring or corrective procedure. He/She will audit the different records, including records for monitoring, calibration and maintenance, training, complaints and reports from clients and control authorities. He will prepare a report that will be submitted to management and discussed during a meeting with management and the HACCP team. The audit exercise will be also used as an opportunity to introduce new procedures, monitoring techniques or critical limits to take into consideration new developments, including new regulatory requirements.

11. Establish verification procedures

Verification is the application of methods, procedures and tests, including random sampling and analysis and other evaluations, in addition to monitoring to determine compliance with the HACCP plan. The objective of verification procedures is to determine if the HACCP system is working effectively.

Careful preparation and implementation of the HACCP plan does not guarantee the plan's effectiveness. Verification procedures are necessary to assess the effectiveness of the plan and to confirm that the HACCP system adheres to the plan.

Verification should be undertaken by an appropriately qualified individual or individuals who are capable of detecting deficiencies in the plan or its implementation.

Verification activities should be documented in the HACCP plan. Records should be made of the results of all verification activities. Records should include methods, dates, individuals and/or organizations responsible, results or findings and actions taken.

12. Establish documentation and record keeping

Records are essential for reviewing the adequacy of the HACCP plan and the adherence of the HACCP system to the HACCP plan. A record shows the process history, the monitoring, the eventual deviation and subsequent corrective actions that occurred at the identified CCP. It may be in any form, e.g. processing chart, written record, computerized record. It is imperative to maintain complete, current, properly filed and accurate records. Failure to document the control of a CCP would be a critical departure from the HACCP plan.

Several types of records should be considered among those relevant in a HACCP program:

- Support documentation for developing the HACCP plan
- Records generated by the HACCP system: Monitoring records of all CCPs
- Deviation and corrective action records, Verification/validation records
- Documentation on methods and procedures used
- Records of employee training programs

Tables 11.2 to 11.4 provide examples of forms to record monitoring different elements of HACCP application in a depuration plant. Other formats can be used to suit specific needs of a given depuration plant as long as they allow capturing the required information.

11.3 TRACEABILITY

Traceability is "*the ability to trace the history, application or location of that which is under consideration*" (ISO 9000:2000). When considering a product, traceability relates to the origin of materials and parts, the processing history and the distribution and location of the product after delivery.

In the case of food safety, the *Codex Alimentarius* (CAC, 2005) defines "*traceability/ product tracing as the ability to follow the movement of a food through specified stages of production, processing and distribution*".

This definition has been further refined into a regulation by the EU to signify "*the ability to trace and follow a food, feed, food producing animal or substance intended to be, or expected to be incorporated in a food or feed, through all stages of production, processing and distribution*" (EU, 2002).

Traceability can use either paper or electronic systems, although most are a mixture of the two. Paper traceability systems are widespread and have been used for a long time throughout the food supply chain. Electronic traceability uses either the bar code systems or the more recent radio frequency identification (RFID) systems. Bar code systems have been in use since the 1970s and are well established in the food industry. RFID technology uses tags that send identification codes electronically to a receiver when passing through a reading area.

Traceability can be divided into *internal* and *external* traceability. *Internal* traceability is traceability of the product and the information related to it, within the company, whereas *external* traceability is product information either received or provided to other members of the food supply chain.

The following information is the minimum required for incoming live shellfish traceability in a depuration plant:

- Name, address and permit number of the harvester
- Date of harvest
- Harvest area and sanitary status (e.g. A, B or C in the EU)
- Shellfish species
- Quantity
- Lot or batch number

In addition, the depurated shellfish may need to trace the following (Figure 11.4):

- Name, address and registration/certification number of depuration plant
- Shellfish specie and quantity

- Depuration date, cycle number or lot number
- Address of place of destination

The traceability records should be kept for a minimum of 90 days (if consumed raw or live) to 1 year for frozen shellfish or longer for canned products.

ALESSANDRO LOVATELLI (FAO)

Figure 11.4: Depurated and packed bivalve products clearly labelled for traceability

Table 11.1: HACCP plan for shellfish depuration*

Critical Control Point(s)	Hazard(s)	Control measure(s)	Critical limit(s)	Monitoring procedure(s)				Corrective action(s)	Record keeping	Verification of records
				What	How	Who	When			
CCP-1 Receiving shellfish	Presence of pathogenic bacteria and viruses in shellfish	Only shellfish from approved harvesting area and delivered by licensed harvester are accepted	Shellfish from unauthorized area or non licensed harvester should not be accepted	License of harvester	Visual verification	Safety supervisor	Each delivery	Identify the affected product and, if feasible, increase the depuration duration. If not, remove product from distribution	Table 11.2	Daily under normal circumstances and at every delivery when a deviation occurs
				Tag accompanying container or transaction record	Visual verification	Safety supervisor	Each delivery	Investigate why the heavily contaminated shellfish was accepted into the plant and deal with the problem		
	Presence of biotoxins in shellfish	Only shellfish from approved harvesting area and delivered by licensed harvester are accepted for depuration	Shellfish from unauthorized area or non licensed harvester should not be accepted	Licence of harvester	Visual verification	Safety supervisor	Each delivery	Identify the product suspect of containing biotoxins and remove it from distribution	Table 11.2	Daily under normal circumstances and at every delivery when a deviation occurs
				Tag accompanying container or bulk shipment transaction record	Visual verification	Safety supervisor	Each delivery	Investigate why the contaminated shellfish was accepted in the plant and deal with the problem		
	Presence of unsafe levels of *Vibrio parahaemolyticus*	Concentrations in harvesting area below statutory or recommended safe limits	Only shellfish from areas deemed to conform to limits are accepted for depuration	Source area of shellfish confirmed as conforming to limits	Visual verification of tag or transaction record	Safety supervisor	Each delivery	Do not accept any shipment with a risk of *Vibrio parahaemolyticus*	Table 11.2	Daily under normal circumstances and at every delivery when a deviation occurs
		Refrigerated shellfish transport	5°C ≤ T ≤ 10 °C Transport duration ≤ 6 hours	Shellfish temperature and transport duration	Temperature measurement and visual verification	Safety supervisor	Each delivery	Do not accept any shipment with a risk of *Vibrio parahaemolyticus*	Table 11.2	Daily under normal circumstances and at every delivery when a deviation occurs
CCP-2 Depuration	Survival of pathogenic bacteria in shellfish	Ensure that water disinfection is operating to design specifications	Depuration design specifications (see chapter 6.2 and manufacturer's specifications)	UV intensity (≥ 10 mW/cm^2/sec)	See chapter 6.2 and manufacturer's specifications	Depuration supervisor	Weekly or as needed	- Identify the affected product and re-depurate. If not possible remove product from distribution - Investigate the cause to bring water disinfection back to operate to design specifications	Table 11.3	Weekly under normal circumstances and at every cycle when a deviation occurs
		Duration	≥ 44 hours	Duration	Timing	Depuration supervisor	Every depuration cycle	- Identify the affected product and re-depurate. If not possible remove product from distribution. - Investigate cause of deviation and deal with it	Table 11.3	Weekly under normal circumstances and at every cycle when a deviation occurs
CCP-3 Storage	Multiplication of surviving bacteria	Refrigerated storage	5°C ≤ T ≤ 10° C	Temperature	Thermometer reading	Safety supervisor	Daily	- Identify the affected product and assess duration of storage at T>10°C. If needed and feasible, re-depurate. If not possible remove from distribution - Investigate cause of deviation and deal with it	Table 11.4	Weekly under normal circumstances and at every cycle when a deviation occurs

* The HACCP plan is provided for illustrative purposes only. Depuration plants operators should adapt it to their specific situation and needs to ensure that the actual hazards and the needed control measures are identified.

Name and address of the company: _____

Name and signature of the manager: _____ Date: _____

Name and signature of the safety supervisor: _____ Date: _____

Table 11.2: Control of shellfish at receiving

Receiving date	Specie and quantity (kg)	Harvest date	Harvest area and area type	Name and licence number of harvester	Duration of transport	Temperature of shellfish at receiving

Name and signature of delivery person: _____ Date: _____

Name and signature of safety supervisor: _____ Date: _____

Table 11.3: Control of shellfish at depuration

Lot number	Date and time in	Date and time out	Quantity	Depuration cycle

Name and signature of depuration supervisor: _____ Date: _____

Name and signature of safety supervisor: _____ Date: _____

Table 11.4: Storage of depurated shellfish

Date in	Lot number	Specie and quantity (kg)	Temperature	Date out

Name and signature of the production manager: _____ Date: _____

Name and signature of safety supervisor: _____ Date: _____

Table 11.5: Recording corrective actions

Date:	Lot:	Critical Control Point:
Description of the control loss (deviation):		
Description of the corrective measure:		
Date and time when control was restored:		
Description of the new situation:		
Name and signature of the production supervisor:		Date:
Name and signature of the safety supervisor:		Date:

Chapter 12

Problem solving

Depuration is a complex process involving a number of interacting variables which affect the activity of the animals and the way that depurated material is taken away from, and kept away from, the shellfish. Table 12.1 gives a number of the common problems that are met together with their possible causes.

More than one problem may be identified at a time and this may help to narrow down the possible causes. When a problem arises, the list of possible causes should be worked through systematically to check whether each applies and thus whether it needs to be rectified. If this approach does not solve the problem(s), help may be available from other operators, industry bodies, fishery officers or local public health officials. Some countries have central technical bodies responsible for assisting the fish and shellfish industry with design and installation of depuration systems (e.g. Seafish in the UK) and/or assisting local authorities with approval of such systems (e.g. Cefas for England and Wales) and these bodies will have specific expertise in this area. The industry bodies, fishery officers or local public health officials should be able to provide contact details for these technical bodies where they exist.

Table 12.1: Common depuration system problems and associated causes

Observed problem	Possible causes
No flow to tank	Blocked inlet pipe Reservoir level too low Blockage or air lock in pipework Wrong valve(s) opened No electrical supply to pump Pump or pump filter blocked
No flow within tank	Blockage or air lock in pipework Wrong valve(s) opened No electrical supply to pump Pump or pump filter blocked
Low flow within tank	Pump inadequately sized for system Pump needs maintenance Partially blocked pump or pump filter Tank drain needs cleaning Pipework needs cleaning Air leak within system Water leak within system
UV lamp not lit	No electrical supply to lamp: switch is off or mains supply faulty, terminals broken or corroded Lamp starter unit needs replacement Lamp broken or faulty
Excessive foaming	Flow-rate too high Water re-used too many times
Shellfish not active	Shellfish unsuitable for depuration (weak, ready to spawn) Shellfish maltreated prior to depuration (physical shock, temperature) Shellfish spawned during depuration Depuration conditions out of recommended range (low dissolved oxygen, salinity, temperature) Water quality poor Excessive water re-use

Table 12.1: Common depuration system problems and associated causes (continued)

Observed problem	Possible causes
Shellfish dead or dying	As above Prolonged period of immersion
Seawater cloudy at time of filling	Water abstracted from too near sea bottom Water abstracted on wrong tidal state Water abstracted after adverse weather conditions Bacterial multiplication in storage system
Seawater becomes cloudy during cycle	Shellfish spawned during depuration Excessive bacterial growth due to shellfish dying in the tank
Seawater *E. coli* ≥ 1/100ml post-UV	Initial level of contamination too high Turbidity too high Ineffective Disinfection: UV lamp(s) not functioning UV lamps efficiency too low Ozone/chlorine concentration too low Contact time too short
Shellfish *E. coli* >230 *E. coli*/100g post depuration (single occasion) >80 *E. coli*/100g post depuration (multiple occasions)	Initial level of contamination too high Shellfish unsuitable for depuration (weak, ready to spawn) Shellfish maltreated prior to depuration (physical shock, temperature) Shellfish spawned during depuration Depuration conditions out of recommended range (low, dissolved oxygen, salinity, temperature) Depuration period too short

Chapter 13

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Appendixes

Appendix 1

Proposed draft code of practice for fish and fishery products *Codex Alimentarius* (29th Session, February 2008) Extracts relevant to live bivalve molluscs

CODEX Codes of Practice provide recommendations that are intended to identify the essential elements necessary for the production of safe food of good quality

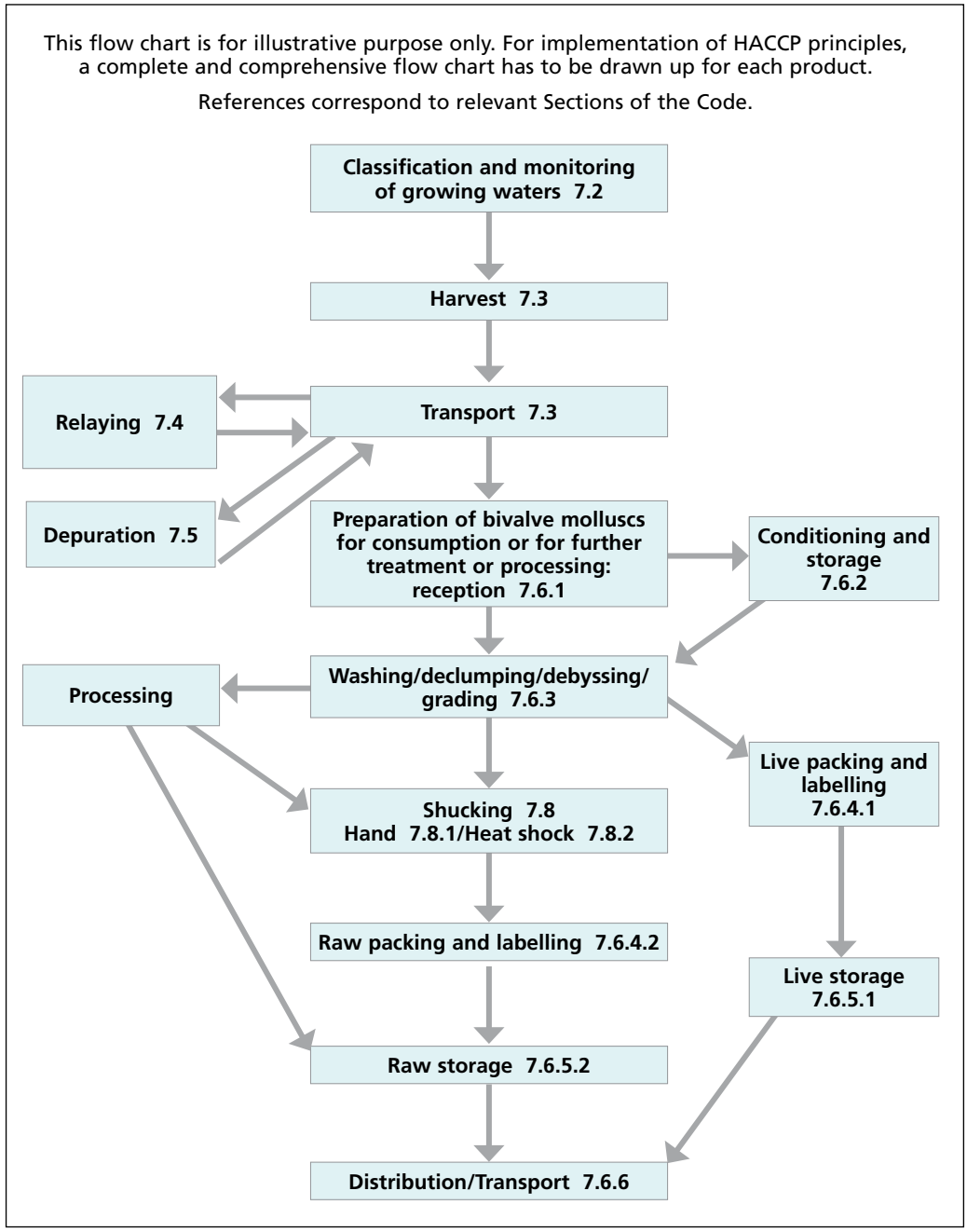
PROPOSED DRAFT CODE OF PRACTICE FOR FISH AND FISHERY PRODUCTS
(At Step 8 of the procedure)
ALINORM 07/30/18
APPENDIX IV

SECTION 2. DEFINITIONS FOR THE PURPOSE OF THIS CODE
2.3 LIVE AND RAW bivalve molluscs

Accepted/ Acceptable/ Approved	means accepted by the official agency having jurisdiction
Conditioning	means placing live bivalve molluscs in tanks, floats or natural sites to remove sand, mud or slime and improve product acceptability
Distribution centre	means any approved on-shore or off-shore installation or establishment for the reception, conditioning, washing, cleaning, grading and packaging of live bivalve molluscs fit for human consumption from which the bivalve molluscs are dispatched alive
Growing areas	means all brackish and marine areas approved for the production or harvesting of bivalve mollusks either by natural growth or by aquaculture destined for human consumption. The growing areas may be approved as production or harvesting areas for bivalve molluscs for direct consumption, or they may be approved as production or harvesting areas for bivalve molluscs for either depuration or relaying
Heat shocking	means the process of subjecting bivalve molluscs in the shell to any form of heat treatment, such as steam, hot water, or dry heat for a short period of time, to facilitate rapid removal of meat from the shell for the purpose of shucking
Depuration	means the reduction of microorganisms to a level acceptable for direct consumption by the process of holding live bivalve molluscs for a period of time under approved, controlled conditions in natural or artificial sea water suitable for the process, which may be treated or untreated
Depuration centre	means any approved establishment for the depuration of live bivalve molluscs
Relaying	means the removal of bivalve molluscs from microbiologically contaminated growing area to an acceptable growing or holding area under the supervision of the agency having jurisdiction and holding them there for the time necessary for the reduction of contamination to an acceptable level for human consumption

SECTION – 7 LIVE AND RAW BIVALVE MOLLUSCS

In the context of recognising controls at individual processing steps, this section provides examples of potential hazards and defects and describes technological guidelines, which can be used to develop control measures and corrective actions. At a particular step only the hazards and defects, which are likely to be introduced or



controlled at that step, are listed. It should be recognised that in preparing a HACCP and/or a Defect Action Plan (DAP) plan it is essential to consult Section 5 which provides guidance for the application of the principles of HACCP and DAP analysis. However, within the scope of this Code of Practice it is not possible to give details of critical limits, monitoring, record keeping and verification for each of the steps since these are specific to particular hazards and defects.

7.1 GENERAL REMARKS, ADDITION TO THE PRE-REQUISITE PROGRAMME

Bivalve molluscs species like oysters, mussels, manilla and hard shell clams can survive for extended periods out of water and can be traded for human consumption as live animals. Other species like cockles can be traded live if carefully handled, but are normally processed. Species not adapted to dry conditions soon die out of water and are best handled as chilled products or processed.

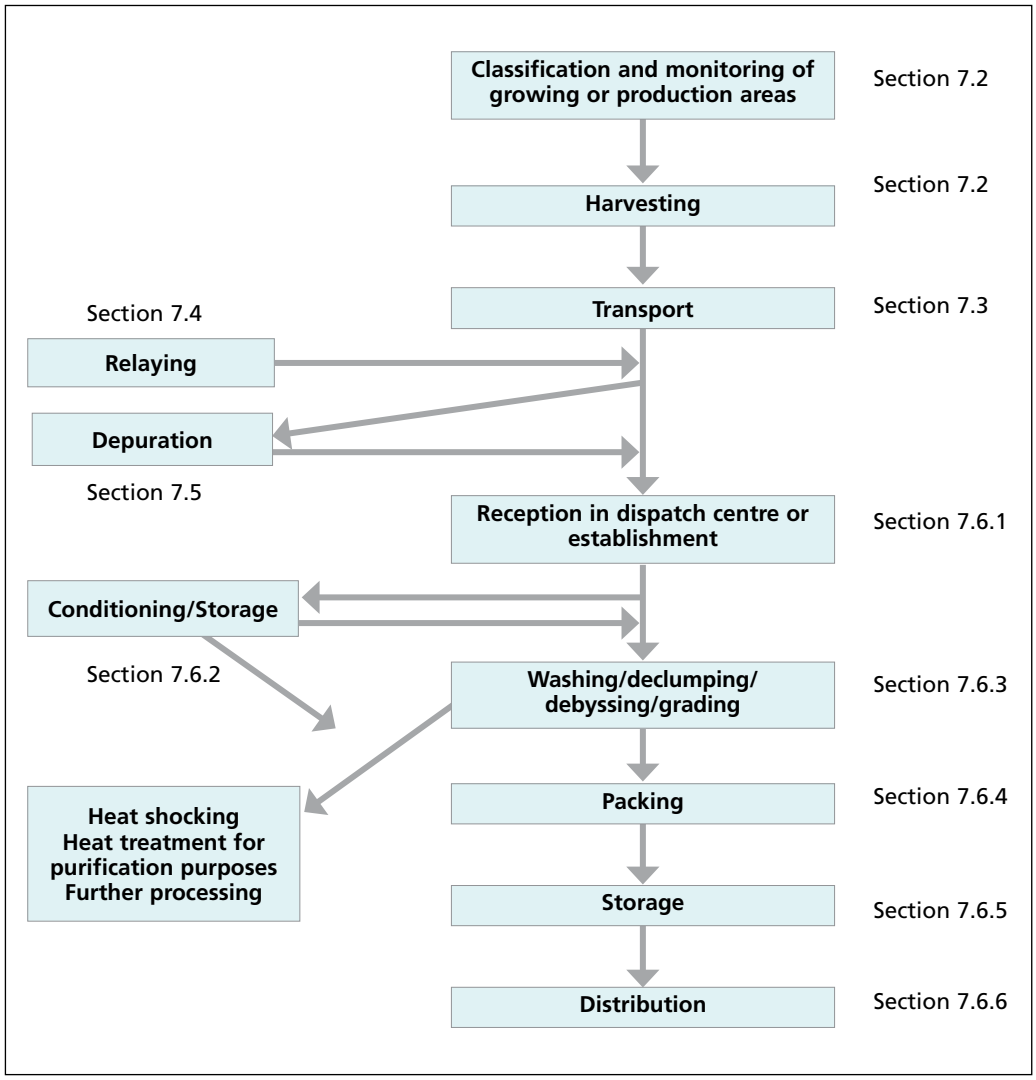


Figure 7.1: Example of a simplified flow diagram for the production of live and raw bivalve molluscs

When spawning (following "gonad ripening") occurs, it becomes undesirable and in many instances impracticable to trade them as live animals. Stress can induce spawning.

The main hazard known for the production of bivalve molluscs is microbiological contamination of waters in which they grow, especially when the bivalve molluscs are intended to be eaten live or raw. Since molluscs are filter feeders they concentrate contaminants to a much higher concentration than the surrounding sea water. The contamination with bacteria and viruses in the growing area is therefore critical for the end product specification and determines the process requirements for further processing. Gastroenteritis and other serious diseases such as hepatitis can occur as result from agricultural run-off and/or sewage contamination like enteric bacterial and/or viral pathogens (norovirus, viruses causing hepatitis) or from natural occurring bacterial pathogens (*Vibrio* spp.). Another hazard is formed by biotoxins. Biotoxins produced by some algae can cause various forms of serious poisoning like diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP) or poisoning caused by Azaspiracid (AZP). Chemical substances, such as heavy metals, pesticides, organochlorides, petrochemical substances may also form a hazard in certain areas.

To control the hazards, identification and monitoring of growing areas is very important for bivalve molluscs safety. The identification, classification and monitoring of these areas is a responsibility for competent authorities in cooperation with fishermen and primary producers. *E. coli*/faecal coliforms or total coliforms may be used as an indicator for the possibility of faecal contamination. If biotoxins are found in the bivalve molluscs flesh in hazardous amounts the growing area must be closed for harvesting bivalve molluscs until toxicological investigation has made clear that the bivalve molluscs meat is free from hazardous amount of biotoxins. Harmful chemical substances should not be present in the edible part in such amounts that the calculated dietary intake exceeds the permissible daily intake.

Bivalve molluscs from waters subject to microbiological contamination, as determined by the authority having jurisdiction, can be made safe by relaying in a suitable area or a depuration process to reduce the level of bacteria if the process is continued long enough, or by processing to reduce or limit target organisms. Depuration is a short-term process commonly used to reduce low levels of bacterial contamination, but long term relaying is required if there is a greater risk of contamination.

Especially when the bivalve molluscs need to undergo relaying or depuration to be eaten raw, stress and excessive shocks of the bivalve molluscs must be avoided. This is important because these bivalve molluscs should be able to function again during depuration, relaying or conditioning.

Many, but not all, species of bivalve molluscs are considered suitable for depuration.

7.2 CLASSIFICATION AND MONITORING OF GROWING AREAS

Potential Hazards:	Microbiological contaminations, Biotoxins, Chemical contamination.
Potential Defects:	Unlikely

Technical Guidance:

There are 5 different types of important hazards coming from the bivalve molluscs growing environment:

- enteric bacterial pathogens (e.g. *Salmonella* spp.);
- enteric viral pathogens (e.g. Norovirus, viruses causing hepatitis);
- naturally occurring bacterial pathogens (e.g. *Vibrio* spp.);
- biotoxins (e.g. okadaic acid group [DSP], saxitoxin group [PSP], brevetoxin group [NSP], domoic acid group [ASP], azaspiracid group [AZP]);
- chemical contaminants (e.g. heavy metals such lead, cadmium and mercury).

7.2.1 Classification of growing areas

Surveys of the growing area, shoreline and land catchment should be conducted to determine sources of both domestic and industrial pollution which may affect the quality of the growing area water and bivalve molluscs. Sources may include municipal sewage outputs, industrial outputs, mine wastes, geophysical contaminants, domestic animal holding pens, nuclear power plants, refineries or other sources. The need to reschedule hygiene surveys will be determined by population shifts and changes in agricultural and industrial activities in the coastal area. Resurveys should be conducted at an acceptable frequency and known pollution sources should be re-evaluated on a regular basis to determine any changes to their impact on the growing area.

When pollution sources have been identified and evaluated, sampling stations for water and/or bivalve molluscs and/or sediments should be established and studies conducted to determine the effects of the pollutants on water and bivalve molluscs quality. The data should be evaluated by the official agency having jurisdiction and growing areas should be classified according to official standards and criteria.

When interpreting growing area data, the official agency having jurisdiction should take into account variations which may affect the level of pollution during the most unfavourable hydrographic and climatic conditions as influenced by rainfall, tides, winds, methods of sewage treatment, population variations and other local factors, since bivalve molluscs respond rapidly to an increase in the number of bacteria or viruses in their environment by accumulating these agents. The agency should also consider that bivalve molluscs have the ability to accumulate toxic chemicals in their tissue in concentrations greater than the levels found in the surrounding water. FAO, WHO, or other international or national food standards may be used as a guide to acceptable levels.

The official agency having jurisdiction should immediately announce decisions concerning the classification of growing areas to the affected producers and depuration and distribution centres.

When sampling shellfish meats for classification purposes, if the limits of any biological or chemical hazard set in the end product specification are exceeded, appropriate measures must be taken under the responsibility of the official agency having jurisdiction.

Classified growing areas should be clearly defined by the official agency having jurisdiction as either:

- suitable for harvesting for direct human consumption, relaying in acceptable water or depuration in an approved depuration centre or approved processing to reduce or limit target organisms; or
- non-suitable for growing or harvesting bivalve molluscs.

7.2.2 Monitoring of growing areas

Growing areas should be routinely monitored for changes in water quality and/or bivalve molluscs quality, and sub-standard areas patrolled to prevent harvesting for purposes other than that established by the official agency.

Biotoxins in bivalve molluscs can be caused by plankton containing toxins. For early warning purposes, where appropriate, it is recommended to have a programme present to monitor growing areas for the species of plankton that can produce toxins and to recognize other environmental signals that a toxic event may be developing.

Harmful chemical substances within bivalve molluscs should not be present in amounts so that the calculated dietary intake exceeds the permissible daily intake. A monitoring system should be present for harmful chemical substances.

When routine monitoring programmes or resurveys show that the growing area no longer meets the classification criteria, the area should be reclassified or closed for harvesting immediately by the official agency having jurisdiction.

In determining the public health suitability of bivalve molluscs classified growing areas the official agency having jurisdiction should consider the following actions:

– Classification/reclassification of growing areas by sanitary survey, monitoring of *E. coli*/faecal coliforms or total coliforms at an appropriate frequency based on the risk of contamination, and other sanitary control measures as applicable.
– Classification/reclassification of growing areas by monitoring of pathogens at an appropriate frequency based on the probability of contamination in bivalve mollusc meat (see 7.2.2.2).
– Closure/reopening of growing areas by the monitoring of biotoxins in bivalve molluscs alone or in combination with the monitoring of phytoplankton in seawater at an appropriate frequency based on the probability of contamination (see 7.2.2.3).
– Control of chemical contaminants.

Under the responsibility of the official agency having jurisdiction the growing areas providing bivalve molluscs for direct human consumption meet the following requirements at time of harvest:
– The area is not subject to contamination that may present an actual or potential hazard to human health.
– The bivalve molluscs harvested meet the end product specification. This can be determined by examination of mollusc's flesh or through adequate monitoring of the water, as appropriate.

Growing areas providing bivalve molluscs for indirect human consumption should be defined in relation to the further procedure of the lot.

7.2.2.1 *E. coli*/faecal coliforms/total coliforms

All growing water and/or molluscan flesh should be monitored for the presence of *E. coli*/faecal coliforms or total coliforms at an appropriate frequency based on the probability and degree of faecal contamination.

Tests for suitable indicator bacteria such as faecal coliforms or *Escherichia coli* or total coliforms should be used to determine the degree of faecal contamination. The effectiveness of indicator bacteria used should be kept under constant review for their reliability as measures for the degree of faecal contamination. If faecal contamination exceeds a certain threshold-levels relaying or depuration for a time approved by the official agency having jurisdiction may be allowed.

E. coli/faecal coliforms or total coliforms may be used as an indicator for the presence of faecal contamination. Because these indicators do not correlate well with the presence of viruses, other controls such as shoreline surveys should always be employed.

Other methods such as bacteriophage and viral detection could also be used as indicators when validated analytical methods become available in the future.

7.2.2.2 Pathogen monitoring

Shellfish sanitation programmes rely upon the use of indicator organisms for the presence of contamination rather than upon attempts to monitor for specific pathogens. However, where there has been a shellfish borne outbreak caused by an identified pathogen such as *Salmonella* and others (*Vibrio* and viruses), monitoring the bivalve molluscs may be appropriate as part of the process of closure/reopening the affected harvest area. The species, and typically the actual strain, should be known to ensure that monitoring is addressing the source of the pathogen. Predetermined acceptance/rejection levels for the pathogen should have been established in order to use such monitoring results for decision making. Other conditions including the sanitary survey requirements should also have been satisfied as a condition of reopening this area.

7.2.2.3 Marine biotoxin control

All growing areas should be monitored for marine biotoxins and/or the presence of algae with potential for producing marine biotoxins at an appropriate frequency based on the risk of contamination. Growing areas should also be monitored for environmental signals that a toxin event maybe occurring, e.g, dead or dying birds, mammals, or fish. The risk of blooms of toxic algae may show seasonal variability and areas may also be affected by toxic algae previously unknown in the surrounding sea or coastal waters. These risks should be recognised when drawing up monitoring schedules. Phytoplankton monitoring is a valuable complementary tool that can be used, in combination with the required monitoring of marine biotoxins in shellfish tissue, to optimize program management and resources.

It is important to note that using indicator shellfish species, the absence of toxicity in indicated species is assumed to imply the absence of toxicity in other species in the growing area. This implication must be verified for each shellfish species and for each group of toxins before defining a particular shellfish species as an indicator for that growing area.

The official agency having jurisdiction should close immediately and effectively patrol affected areas when acceptable levels are exceeded in edible portions of bivalve molluscs meats. These areas should not be opened before toxicological investigation has made clear that the bivalve molluscs meat is free from hazardous amounts of biotoxins. The official agency having jurisdiction should immediately announce these decisions to the affected producers and depuration and distribution centres.

In establishing sampling programme over space and time, consideration should be given to assuring adequate location and number of sampling sites. Testing for a particular biotoxin may not be appropriate when it has been demonstrated that this biotoxin has not been associated with bivalve molluscs in the growing and harvesting areas. Sampling frequency must be sufficient to address spatial-temporal changes in micro-algae, toxins in shellfish and to cover the risks of rapid rises in shellfish toxicity.

Spatial Representational Sampling

The selection of sampling stations for both benthic and suspended culture should be based on sites which have historically presented toxicity in the early stages of a toxic event. It is recognised that sampling, generally, cannot be carried out in a statistically valid way without excessive cost. In order to protect public health, the selection of sampling stations should give appropriate coverage of the extent of a toxic event or the likely "worst case scenario" in a growing area. This should be based on expert judgment using the following factors:

- Hydrography, known upwellings, fronts, current patterns and tidal effects.
- Access to sampling stations in all weather conditions during harvesting.
- Desirability of toxin and micro-algal sampling at the same sampling station.
- In addition to primary (routine) stations, the need for secondary (complementary) and offshore stations.
- Existence of *in-situ* growth (for example, toxic micro-algae from cyst beds).
- The advection of offshore toxic micro-algal blooms into growing areas.

Routine sampling for micro-algae will generally mean taking an integrated sample from the water column. When a toxic event is in progress or developing, targeted, depth-specific sampling should be considered.

Sampling for shellfish grown in suspension, should at least involve an integrated sample composed of shellfish taken from the top, middle and bottom of the lines.

Temporal Representational Sampling
Minimum weekly sampling frequencies are adopted by most monitoring programmes in areas where toxicity is prevalent and where harvesting is taking place or about to take place. Decisions on the frequency of sampling should be based on risk evaluation. Inputs into the decision may include factors such as seasonality (toxicity and/or harvesting), accessibility, historical baseline information, including toxin and micro-algal data, and the effects of environmental factors such as wind, tide and currents.

Sampling frequency and the factors that may lead to it being changed should be described in a "Marine Biotoxin Action Plan" for the growing area.

Shellfish Sample Size
There is no internationally agreed sample size for different shellfish species. There may be high variability of toxicity among individual shellfish. The number of shellfish sampled should be sufficient to address this variability. For this reason, the number of shellfish in the sample, rather than the mass of the shellfish flesh should be the determining factor for the sample size. Additionally, the size of the sample should be sufficient to allow the test or tests for which the sample is being taken to be carried out, and the shellfish sampled should be of the size marketed.

7.2.2.4 Marine biotoxin test methods
Methods suitable for the determination of marine biotoxines are listed in the draft Standard for Live and Raw Bivalve Molluscs. Any methods may be deemed suitable for screening purposes provided they are approved by a country's competent authority.

7.2.2.5 Chemical contaminants
Growing areas should be monitored for chemical contaminants on a sufficiently frequent basis to provide confidence that any identified sources of chemical contamination are not contaminating the shellfish. Shellfish growing areas where there are no known point sources of likely chemical contamination should only require occasional checks every few years. However, where there are known point sources of specific contamination shellfish may need to be checked more frequently on a routine basis. There should also be the capacity to sample shellfish reactively if a defined event occurs – for example a spillage of anti-fouling paint.

7.3 HARVESTING AND TRANSPORTATION OF LIVE BIVALVE MOLLUSCS

Refer also to Sections 3.1, 3.3, 3.4 and 3.5

This section applies to the transportation of bivalve molluscs for the purpose of direct human consumption, relaying, depuration, processing to reduce or limit target organisms, or further processing.

Appropriate handling procedures depend on different species, growing area and season.

Potential Hazards:	Microbiological contaminations, Biotoxins, Chemical contamination.
Potential Defects:	Physical damage

Technical Guidance:

Dredges and other harvesting equipment, decks, holds and containers, which are contaminated from use in a polluted area, should be cleaned and if applicable disinfected (sanitized) before being used for bivalve molluscs from an unpolluted area.

- Holds in which bivalve molluscs are held or containers should be so constructed that the bivalve molluscs are held above the floor level and drained so that the bivalve molluscs is not in contact with wash-down or bilge water, or shell fluid. Where necessary a bilge pumping system must be provided.
- Suitable precautions should be taken to protect bivalve molluscs from being contaminated by polluted water, droppings from sea birds, footwear which may have been in contact with faecal matter or by other polluted material. No overboard discharge of waste, including human faecal material, should occur from harvest vessels around shellfish growing areas. No animals should be allowed on harvest vessels.
- Wash-down pumps should draw water only from non-contaminated seawater.
- Bivalve molluscs should be harvested from and stored in an growing area or relaying area acceptable to the official agency having jurisdiction.
- On removal from water or during handling and transportation, bivalve molluscs should not be subjected to extremes of heat or cold or sudden variations in temperature. Temperature control is critical in handling live bivalve molluscs. Special equipment, such as insulated containers and refrigeration equipment should be used if prevailing temperatures and the time involved so require. Bivalve molluscs should not be exposed to full sun or surfaces heated by the sun or come into direct contact with ice and other freezing surfaces, nor should it be held in closed containers with solid carbon dioxide. In most cases storage above 10°C (50°F) or below 2°C (35°F) should be avoided.
- Bivalve molluscs should be freed from excessive mud and weed soon after being harvested by washing it with clean seawater or potable water under suitable pressure. Wash water should not be allowed to flow over bivalve molluscs already cleaned. The water could be re-circulated if it meets the definition for clean water.
- The interval between harvesting and immersion in water for relaying, storage, conditioning or depuration should be kept as short as possible. This also applies to the interval between final harvesting and handling in a distribution centre.
- If bivalve molluscs are to be re-immersed after harvest they should be re-immersed in clean seawater.
- Appropriate documentation should be maintained for harvesting and transportation activities.

7.4 RELAYING

The requirements for classification and monitoring of growing areas also apply to Relaying areas.

Relaying is intended to reduce the level of biological contaminants that may be present in bivalve molluscs which have been harvested from contaminated areas to such levels that the bivalve molluscs will be acceptable for human consumption without further processing. Bivalve molluscs harvested for relaying should only be harvested from areas that are so designated/classified by the official agency having jurisdiction. Relaying methods vary worldwide. Bivalve molluscs may be placed in floats, rafts or directly on the bottom.

Potential Hazards:	Microbiological contaminations, Biotoxins, Chemical contamination.
Potential Defects:	Unlikely

Technical Guidance:

- Relaying operations should be strictly supervised by the official agency having jurisdiction to prevent contaminated bivalve molluscs from being diverted directly to the consumer market or from cross contamination of other bivalve molluscs. Boundaries of relaying areas should be clearly identified by buoys, poles or other fixed means. These areas should be adequately separated from the bivalve molluscs in adjacent waters and suitable control systems should be in place to prevent cross contamination and commingling.
- Holding time and minimum temperature in the accepted area prior to harvest will be determined by the official agency having jurisdiction according to the degree of contamination before relaying, the temperature of the water, the bivalve molluscs species involved and local geographic or hydrographic conditions to ensure that contamination levels have been adequately reduced.
- Relaying sites could become biotoxic from a bloom, or could become an unexpected a source of environmental pathogens such as vibrio bacteria, and should therefore be monitored as appropriate while they are being used for relaying.
- Bivalve molluscs should be laid out at a density which will permit them to open and undergo natural depuration.
- Appropriate documentation should be maintained for relaying operations.

7.5 DEPURATION

Refer also to Sections: 3.2, 3.3, 3.4 and 3.5

Depuration is intended to reduce the number of pathogenic micro-organisms that may be present in bivalve molluscs which have been harvested from moderately polluted areas to such levels that the bivalve molluscs will be acceptable for human consumption without further processing. Depuration alone is not suitable for cleansing bivalve molluscs from more heavily contaminated areas or areas subject to contamination by hydrocarbons, heavy metals, pesticides, viruses, vibrios or biotoxins. Bivalve molluscs harvested for depuration should only be harvested from areas that are so designated/ classified by the official agency having jurisdiction.

The required conditions vary according to the species of molluscs and the design of the depuration system.

For natural functioning and therefore depuration to occur it is essential that the molluscs have not been over-stressed or damaged during harvesting or handling prior to depuration and should not be in a seasonally weak or spawning condition.

Depuration centres should maintain the same hygiene standards as sections 3.2, 3.3, 3.4, 3.5.

Potential Hazards:	Microbiological contaminations
Potential Defects:	Physical damage

Technical Guidance:

Depuration centres and tanks should be approved by the official agency having jurisdiction.

- Bivalve molluscs subjected to the depuration process should not contain metallic ions, pesticides, industrial wastes or marine biotoxins in such quantities that it presents a health hazard to the consumer.
- Use only shellstock designated as acceptable by the official agency having jurisdiction.

- The process and the equipment, e.g. tanks, used for depuration should be acceptable to the official agency having jurisdiction.
- Dead or damaged bivalve molluscs should be removed before the depuration process, when practicable. Surfaces of shells should be free from mud and soft commensal organisms. If necessary the bivalve molluscs should be washed with clean sea water before the depuration process.
- The length of the period of depuration should be adapted to the water temperature and physical water quality parameters (clean sea water, salinity, dissolved oxygen and pH levels suitable to permit the bivalve molluscs to function normally), the degree of contamination before depuration and the bivalve molluscs species. Microbiological investigation of process water and of bivalve molluscs meat should be used to assess depuration parameters. It should be taken into account that viruses and *Vibrio* spp. are more persistent during depuration than the indicator bacteria mostly used for microbiological monitoring and that the reducing of the number of indicator bacteria does not always reflect the real situation as regards contamination by viruses and *Vibrio*.
- Water used in depuration tanks should be changed continuously or at suitable intervals or if recirculated be treated properly. The flow of water per hour should be sufficient to the amount of bivalve molluscs treated and should depend on the degree of contamination of the bivalve molluscs.
- Bivalve molluscs undergoing depuration should remain immersed in clean sea water until it satisfies the sanitary requirements of the official agency having jurisdiction.
- Bivalve molluscs should be laid out at a density which will permit them to open and undergo natural depuration.
- During the process of depuration, the water temperature should not be allowed to fall below the minimum at which bivalve molluscs remain physiologically active; high water temperatures which adversely affect the pumping rate and the depuration process should be avoided; tanks should be protected from the direct rays of the sun when necessary.
- Equipment in contact with water, i.e. tanks, pumps, pipes or piping, and other equipment should be constructed of non-porous, non-toxic materials. Copper, zinc, lead and their alloys should preferably not be used in tanks, pumps or piping systems used in depuration processing.
- To avoid recontamination of bivalve molluscs undergoing depuration, unpurified bivalve molluscs should not be placed in the same tank as bivalve molluscs which are already undergoing depuration.
- On removal from the depuration system, bivalve molluscs should be washed with running potable water or clean sea water, and handled in the same manner as living bivalve molluscs taken directly from a non-polluted area. Dead, with broken shells or otherwise unwholesome bivalve molluscs should be removed.
- Before removing the bivalve molluscs form the tanks drain the water from the system to avoid resuspension and reingestion. The tanks should be cleaned after each use and disinfected at suitable intervals.
- After depuration the bivalve molluscs should meet the end product specification.
- Appropriate documentation should be maintained for depuration.

7.6 PROCESSING OF BIVALVE MOLLUSCS IN A DISTRIBUTION CENTRE OR AN ESTABLISHMENT

Some countries require that bivalve molluscs that are to be frozen and/or shucked, and/or processed to reduce or limit target organisms must first pass through a "distribution centre" from which they exit alive. Other countries allow freezing, shucking, and

processing to reduce or limit target organisms to occur in establishments that perform the functions of a "distribution centre." Both practices are legitimate and the products from each one should be equally permitted in international trade. Where "distribution centre" activities and processing activities occur under the same roof, care must be taken to ensure adequate separation of activities to prevent cross-contamination or commingling products.

Distribution centres that prepare live bivalve molluscs suitable for direct consumption and establishments that prepare live and raw bivalve molluscs suitable for direct consumption should maintain the same hygiene standards as sections 3.2, 3.3, 3.4, 3.5.

7.6.1 Reception

Potential Hazards:	Microbiological, chemical and physical contamination
Potential Defects:	Viable parasites ,physical damage, foreign matter, dead or dying of bivalve molluscs

Technical Guidance:

- Stress and excessive shocks to bivalve molluscs that will be dispatched live from a distribution centre or other establishment must be avoided.
- Distribution centres and other establishments that prepare live bivalve molluscs should only accept bivalve molluscs which meet the end product specification and which originate directly from approved growing areas or after relaying in an approved relaying area or after depuration in an approved depuration centre or tank.

7.6.2 Conditioning and storage of bivalve molluscs

Refer also to Sections 3.2, 3.3, 3.4 and 3.5

Potential Hazards:	Microbiological contamination, chemical contamination, biotoxins
Potential Defects:	Physical damage, foreign matter, dead or dying of bivalve molluscs

Technical Guidance:

Conditioning means storage of bivalve molluscs in sea water tanks, basins, floats, rafts or natural sites with the intention to remove mud, sand and slime.

- The process of storing bivalve molluscs in sea water tanks, basins, floats, natural sites or rafts can be used if it is acceptable to the official agency having jurisdiction.
- Only clean sea water should be used in the tanks, floats, natural sites or rafts and should be of an adequate salinity and adequate physical water quality parameters to permit the bivalve molluscs to function normally. Optimum salinity will vary with bivalve molluscs species and with the harvesting area. Water condition has to be satisfactory adequate for the process. Where natural sites are used for conditioning these should be classified by the official agency having jurisdiction.
- Before conditioning or storage bivalve molluscs should be washed to remove mud and soft commensal organisms and dead or damaged bivalve molluscs should be removed when practicable.
- During storage bivalve molluscs should be laid out at a density and under such conditions that will permit them to open and function normally.

- The oxygen content in the seawater should be maintained at an adequate level at all times.
- The temperature of the water in storage tanks should not be allowed to rise to such levels as to cause weakness of the bivalve molluscs. If ambient temperatures are excessively high, tanks should be placed in a well-ventilated building or away from the direct rays of the sun. The length of the period of conditioning should be adapted to the water temperature.
- Bivalve molluscs should be stored in clean sea water only for such time as they remain sound and active.
- Tanks should be drained, cleaned and disinfected at suitable intervals.
- Recirculating wet storage systems must contain approved water treatment systems.

7.6.3 Washing, declumping, debyssing and grading

Refer also to Sections 3.2, 3.3, 3.4 and 3.5

Potential Hazards:	Microbiological contamination, Chemical and Physical contamination
Potential Defects:	Mechanical damage

Technical Guidance:

- All steps in the process, including packaging, should be performed without unnecessary delay and under conditions which will prevent the possibility of contamination, deterioration and the growth of pathogenic and spoilage micro-organisms.
- Damage to shells and stress will shorten the shelf life of bivalve molluscs and increase the risk of contamination and deterioration. So bivalve molluscs have to be handled carefully:
  – The number of handlings with bivalve molluscs should be minimised;
  – Excessive shocks should be avoided.
- The different process steps should be supervised by technically competent personnel.
- The outsides of the shells should be washed free of mud, and all soft adhering organisms should be removed. Hard adhering organisms should also be removed when possible, care being taken not to chip lips of shells by vigorous washing. Washing should be carried out using pressurised clean (sea) water.
- Bivalve molluscs having formed clumps should be declumped and debyssed as appropriate. The equipment used should be designed and adjusted to minimise the risk of damage to the shells.

7.6.4 Packing and Labelling

Refer also to Sections: 3.2, 3.3, 3.4 and 3.5

All steps in the process of packaging should be performed without unnecessary delay and under conditions that will prevent the possibility of contamination, deterioration and the growth of pathogenic and spoilage micro-organisms.

The packaging material should be appropriate for the product to be packed and for the expected conditions of storage and should not transmit to the product harmful or other objectionable substances or odours and tastes. The packaging material should be sound and should provide appropriate protection from damage and contamination.

7.6.4.1 Packing and Labelling of Live Bivalve Molluscs

Potential Hazards:	Microbiological contamination, physical contamination, chemical contamination
Potential Defects:	Incorrect labeling, presence of damaged or dead bivalve molluscs, foreign matter

Technical Guidance:

- Before packing bivalve molluscs should undergo visual inspection. Bivalve molluscs which are dead, with broken shells, with adhering soil or otherwise unwholesome, should not be passed for human consumption.
- The packaging material should avoid contamination and should be drained.
- Labels should be clearly printed and must comply with the labeling laws of the country where the product is marketed. The packaging material may be used to bear an indication as to how the bivalve molluscs should be kept from the time they were bought at the retailer. It is recommended to include the date of packaging.
- All packaging material should be stored in a clean and sanitary manner. Product containers should not have been used for any purpose, which may lead to contamination of the product. Packaging material should be inspected immediately before use to ensure that they are in a satisfactory condition and where necessary disposed of or cleaned and/or disinfected; when washed they should be well drained before filling. Only packaging material required for immediate use should be kept in the packing or filling area.

7.6.4.2 Packing and Labelling of Raw Bivalve Molluscs

Potential Hazards:	Microbiological contamination, physical contamination, chemical contamination
Potential Defects:	Incorrect labeling, presence of damaged or dead bivalve molluscs, foreign matter

Technical Guidance:

- Labels should be clearly printed and must comply with the labeling laws of the country where the product is marketed. The packaging material or label may be used as a means to convey appropriate storage instructions to the consumer after retail purchase. It is recommended to include the date of packaging
- All packaging material should be stored in a clean and sanitary manner. Only packaging material required for immediate use should be kept in the packing or filling area.
- Shucked and post harvest treated product should be packed and chilled or frozen as soon as possible.
- Freezing should take place quickly (see Section 8.3). Slow freezing will damage meat.
- If labels on post harvest treated raw bivalve molluscs make safety claims relating to the post harvest treatment, the claims should be specific to the target hazard that has been eliminated or reduced.

7.6.5 Storage

7.6.5.1 Storage of Live Bivalve Molluscs

Potential Hazards:	Microbiological contamination
Potential Defects:	Physical damage

Technical Guidance:

- The end product should be stored under such conditions as will preclude the contamination with and/or proliferation of micro-organisms. The packaging material of the end product should not have direct contact with the floor but should be placed on a clean, raised surface.
- Storage periods should be kept as short as possible.
- Reimmersion in or spraying with water of live bivalve molluscs must not take place after they have been packed and have left the distribution centre except in the case of retail sale at the distribution centre.

7.6.5.2 Storage of Raw Bivalve Molluscs

Potential Hazards:	Microbiological contamination, chemical and physical contamination
Potential Defects:	Physical damage

Technical Guidance:

- Storage periods should be kept as short as possible
- Damage to packaging of frozen product should be avoided.

7.6.6 Distribution/Transport

7.6.6.1 Distribution of Live Bivalve Molluscs

Refer also to Sections 3.6 and 17

Potential Hazards:	Microbiological contamination
Potential Defects:	Physical damage

Technical Guidance:

- The product should be dispatched in the sequence of the lot numbers.
- Temperature should be maintained during distribution to control microbial growth.
- Bivalve molluscs intended for human consumption should only leave the distribution centre in closed packaging.
- The means of transport should provide sufficient protection of the bivalve molluscs against damage to the shells from shocks. The bivalve molluscs should not be transported with other products which might contaminate them.

7.6.6.2 Distribution of Raw Bivalve Molluscs

Potential Hazards:	Microbiological contamination
Potential Defects:	Unlikely

Technical Guidance:

- Temperature should be maintained during distribution to control microbial growth.
- The product should be dispatched in the sequence of the lot numbers.
- Transportation should be able to maintain chilled or frozen product for safety and quality."

7.7 PROCESSING TO REDUCE OR LIMIT TARGET ORGANISMS

Refer also to Sections 3.2, 3.3, 3.4, and 3.5.

Post harvest treated bivalve molluscs are products prepared from live or raw bivalve molluscs that have been treated after harvest to eliminate, reduce or limit specified target organisms within the product to levels that are satisfactory to the official agency having jurisdiction. Post harvest treatment is intended to retain the sensory qualities of a live bivalve mollusc. As with all live and raw bivalve molluscs, post harvest treated bivalve molluscs must meet all microbiological criteria associated with traditional harvest water controls designed to prevent faecal contamination and resulting introduction of enteric pathogens as well as toxins and other contaminants. However, these growing area controls are not designed for control of pathogens that are independent from faecal contamination. These treatments may include the application of low heat, hydrostatic pressure (e.g. 60K lb/6 min.) irradiation, and individual quick freezing.

Potential Hazards:	Microbiological contamination
Potential Defects:	Coagulation of meat, defective meat texture, hydrostatic medium forced into the flesh.

Technical Guidance:

- Any treatment developed to eliminate or reduce pathogens should be thoroughly validated scientifically to ensure that the process is effective (see the Draft Guidelines for the Validation of Food Safety Control Measures).
- The control treatments (heat, pressure, etc.) should be closely monitored to ensure that the product does not undergo textural changes in the flesh that are unacceptable to the consumer.
- The treatment parameters established to reduce or limit pathogens should be approved by the official agency having jurisdiction.
- Each establishment which purifies bivalve molluscs with a heat treatment must develop a heat treatment process schedule, acceptable to the official agency having jurisdiction, which addresses such critical factors as the species and size of bivalve molluscs, time of exposure to heat, internal bivalve molluscs temperature, type of heat process used, water/steam to bivalve molluscs ratios, nature of heat equipment, measurement devices and their calibration, post heating chilling operations, cleaning and sanitising of heat process equipment.

7.8 SHUCKING

Shucking is the processing step that removes the edible portion of the mollusc from the shell. It is usually done by hand, mechanically or through heat shock with steam or hot water. This step may expose the product to microbiological or physical contamination.

7.8.1 Hand and mechanical shucking and washing

Physical removal of shellfish meat from the shell will often expose the product to dirt, mud and detritus that should be removed before further processing through washing or other means.

Potential Hazards:	Physical contamination, microbiological contamination
Potential Defects:	Cuts and tears of the flesh, presence of sand and mud

Technical Guidance:

- Care should be taken to eliminate excess mud, detritus and sand from the shucking tables.
- The product should be examined to ensure that cuts and tears are minimized.
- Shucked molluscs should be rinsed or washed to further eliminate mud, sand, detritus and reduce the microbiological level of the product.

7.8.2 Heat shocking of bivalve molluscs followed by packing

Heat shocking is a method to remove shells from the bivalve molluscs.
Refer also to Sections 3.2, 3.3, 3.4 and 3.5

Potential Hazards:	Physical contamination
Potential Defects:	Unlikely

Technical Guidance:

- The bivalve molluscs must come from approved growing areas and/or after relaying in an approved relaying area or depuration in an approved depuration centre or tank. Each establishment which heat shucks bivalve molluscs should develop a heat shuck process schedule, acceptable to the official agency having jurisdiction, which addresses such critical factors as the species and size of bivalve molluscs, time of exposure to heat, internal bivalve molluscs temperature, type of heat process used, water/steam to bivalve molluscs ratios, nature of heat equipment, measurement devices and their calibration, post heating chilling operations, cleaning and sanitising of heat process equipment.
- All bivalve molluscs should be washed with pressurized potable water or clean sea water and culled for damaged and dead bivalve molluscs prior to heat treatment.
- Before heat shocking the bivalve molluscs should be inspected to determine whether the bivalve molluscs are alive and not badly damaged.
- Heat shocked bivalve molluscs should be cooled to 7°C or less within two hours of being heat treated (this time includes the shucking process). This temperature should be maintained during transport, storage and distribution.
- The heat shocked bivalve molluscs should be packed as soon as possible. Before packing the bivalve molluscs should be examined for objectionable matter such as shell pieces.

7.9 DOCUMENTATION

- The transport of live bivalve molluscs from a growing area to a distribution centre, depuration centre, relaying area or establishment should be accompanied by documentation for the identification of batches of live bivalve molluscs.
- Storage and transport temperatures should be indicated.

- Permanent, legible and dated records of relaying and depuration should be kept concerning each lot. These records should be retained for a period of minimal one year.
- Depuration centres or tanks and distribution centres and establishments should only accept lots of live bivalve molluscs with documentation issued by or accepted by the official agency having jurisdiction. Where appropriate, this document should contain the following information
  – the gatherer's identity and signature;
  – the date of harvesting;
  – common and/or scientific name and quantity of bivalve molluscs;
  – the location of the growing area and the status of this area (suitable for harvesting for direct human consumption, suitable for relaying, suitable for depuration, suitable for approved processing to reduce or limit target organisms).
  – for distribution centres and establishments, if appropriate, the date and duration of depuration and the responsible's identity and signature.
  – for distribution centres and establishments, if appropriate, the date and duration of relaying, the location of the relaying area and the responsible's identity and signature.
- Complete records of harvest area and date of harvest and length of time of relaying or depuration of each lot should be maintained by the distribution centre or establishment for a period designated by the official agency having jurisdiction.

7.10 LOT IDENTIFICATION AND RECALL PROCEDURES

Refer also to Section 3.7

- "Each product should have an easy identifiable lot number. This lot number must include an identification code, the number of the establishment that distributes the product, the country of origin and day and month of packing, in order to facilitate the tracing/traceability of the product. A record keeping system should be based on these lot numbers so that individual lots of bivalve molluscs can be traced from the growing area to the end user".

Appendix 2

Proposed draft standard for live bivalve molluscs and for raw bivalve molluscs processed for direct consumption or for further processing. *Codex Alimentarius*, Committee on Fish and Fishery Products (29th Session, February 2008)

(At Step 8 of the procedure)
ALINORM 07/30/18
APPENDIX V

1 SCOPE

This standard applies to live bivalve molluscs and to raw bivalve molluscs that have been shucked and/or frozen, and/or processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs. Raw bivalve molluscs are marketed either in a frozen or chilled state. Both live and raw bivalve molluscs may be intended for direct consumption or further processing. The standard does not apply to scallops when the final product is the adductor muscle only.

Part I below applies to live bivalve molluscs while Part II applies to raw bivalve molluscs.

PART I – LIVE BIVALVE MOLLUSCS

I-2 DESCRIPTION

I-2.1 Product Definition

Live bivalve molluscs are products that are alive immediately prior to consumption. Presentation includes the shell.

I-2.2 Process Definition

Live bivalve molluscs are harvested alive from a harvesting area either approved for direct human consumption or classified to permit harvesting for an approved method of purification, e.g. relaying or depuration, prior to human consumption. Both relaying and depuration must be subject to appropriate controls implemented by the official agency having jurisdiction.

I-2.3 Presentation

Any presentation of the product shall be permitted provided that it:

- meets all requirements of this standard; and
- is adequately described on the label to avoid confusing or misleading the consumer.

The bivalve molluscs may be packed by weight, count, count per unit of weight, volume or per package.

I-3 ESSENTIAL COMPOSITION AND QUALITY FACTORS

I-3.1 Bivalve Molluscs

Live bivalve molluscs should possess organoleptic characteristics associated with freshness, as well as an adequate response to percussion (i.e. the shellfish will close by themselves when tapped) and freedom from extraneous matter, as determined by specialists familiar with the species concerned.

I-3.2 Ice for Packing

If ice is used for packing, the water should be made from potable water or clean seawater.

I-3.3 Final Product

Live bivalve molluscs shall meet the requirements of this standard when lots examined in accordance with Section I-9 comply with the provisions set out in Section I-8. Live bivalve molluscs shall be examined by the methods given in Section I-7.

I-4 FOOD ADDITIVES

Food additives are not permitted in live bivalve molluscs.

I-5 CONTAMINANTS

I-5.1 The products covered by this Standard shall comply with the Maximum Levels of the Codex General Standard for Contamination and Toxins in Foods (CODEX/ STAN 193-1995) and the maximum residue limits for pesticides and veterinary drugs established by the CAC.

I-5.2 The following provisions apply to the edible parts of live bivalve mollusc (the whole part or any part intended to be eaten separately).

Name of biotoxin groups	Maximum level/kg of mollusc flesh
Saxitoxin (STX) group	≤0.8 miligrams (2HCL) of saxitoxin equivalent
Okadaic acid (OA) group	≤0.16 miligrams of okadaic equivalent
Domoic acid (DA) group	≤20 miligrams domoic acid
Brevetoxin (BTX) group	≤200 mouse units or equivalent
Azaspiracid (AZA) group	≤0.16 miligrams

I-6 HYGIENE AND HANDLING

I-6.1 It is recommended that the products covered by provisions of this standard be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1 – 1969), the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003) and other relevant Codex texts such as Codes of Hygienic Practice and Codes of Practice.

I-6.2 The products should comply with any microbiological criteria established in accordance with the Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1997).

I-6.3 Growing area monitoring programs, irrespective of the type of indicator bacteria used, must ensure that live bivalve molluscs destined for direct human consumption meet the *E. coli* limit as identified below when tested in accordance with an MPN method specified in ISO 16649-3 or equivalent.

I-6.4 In analysis involving five (5) 100g samples of the edible parts (the whole part or any part intended to be eaten separately), none may contain more than 700 *E. coli* and not more than (1) of five (5) samples may contain between 230 and 700 *E. coli*, or equivalent as decided by the competent authority having jurisdiction.

Escherichia coli/100g n=5 c=1 m=230 M=700

where "n" = the number of sample units, "c" = the number of sample units that exceed the limit "m", and "M" is the limit wich no sample unit may exceed.

I-6.5 In analysis involving five (5) 25g samples of the edible parts (the whole part or any part intended to be eaten separately), no sample may indicate the presence of *Salmonella* when tested using a method validated against the reference method ISO 6579.

I-6.6 Where the microbiological criteria are not met, actions should be taken as deemed appropriate by the competent authority. In following up, consideration should be given to detention, recall and further processing in a manner to eliminate the hazard from implicated lots. In addition, assessement of the status of harvesting areas and/or establishment controls should be undertaken.

I-7 LABELLING

In addition to the provisions of the Codex General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985) the following specific provisions apply:

I-7.1 The Name of the Food

The name of the food to be declared on the label shall be the common or usual name of the species of bivalve molluscs in accordance with the law and custom of the country in which the food is sold and in a manner not to mislead the consumer.

I-7.1.1 There shall appear on the label, reference to the presentation provided for in Section I-2.3-Presentation in close proximity to the name of the product in such descriptive terms that will adequately and fully describe the nature of the presentation of the product to avoid misleading or confusing the consumer.

I-7.1.2 In addition to the specified labelling designations above, the usual or common trade names of the variety may be added so long as it is not misleading to the consumer in the country in which the product will be distributed.

I-7.2 Content Declaration

Live bivalve molluscs shall be labelled by weight, count, count per unit weight, or volume as appropriate to the product.

I-7.3 Storage Instructions

The label shall specify the conditions for storage and/or temperature that will maintain the product safety/viability during transportation, storage and distribution.

I-7.4 Labelling of Non-retail Containers

Labelling for live bivalve molluscs shall contain the following information:

(i) Identification of the product by common and/or scientific names as determined by the competent authority. The country where the product is sold can determine if the scientific name must be indicated on the label.
(ii) Information that might be needed in the event of a food safety problem, such as lot identification which could be lot code or date and location of harvest, information about harvest area, date of harvesting, purification or relaying as appropriate, as well as identification of the despatch centre or other establishment from which they were shipped.
(iii) Durability or shelf life.

Date of minimum durability may be replaced by the statement "Bivalves must be alive when sold".

I-8 SAMPLING, EXAMINATION AND ANALYSES

I-8.1 Sampling

(i) Sampling of lots for examination of the product shall be in accordance with the Codex General Guidelines on Sampling (CAC/GL 50-2004)

(ii) Each sample shall contain a sufficient number of bivalves molluscs to ensure that the sample is representative.

(ii) The portion of the bivalve mollusc analysed should be the edible part. This is generally the whole tissue. Where whole-tissue analysis is not possible or practical, the most contaminated tissue (e.g. the digestive gland) may be dissected and analysed and the results converted to an edible tissue basis. The conversion factor should be supported by adequate data.

I-8.2 Sensory and Physical Examination

Samples taken for sensory and physical examination shall be assessed by persons trained in such examination and in accordance with procedures elaborated in Sections I-7.3 through I-7.5, and Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories" (CAC/GL 31-1999).

I-8.3 Determination of Count per Unit Weight or Volume

When declared on the label, the count of bivalve molluscs shall be determined by counting the numbers of bivalve molluscs in the container or a representative sample thereof and dividing the count of bivalve molluscs by the actual weight/volume to determine the count per unit weight or volume.

I-8.4 Methods of Analysis of *Escherichia coli* in bivalve molluscs

The ISO/TS 16649-3. Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli – Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide or other validated methods in accordance with the protocol set out in the ISO 16140 or other internationally accepted similar protocol.

I-8.5 Determination of Biotoxins

Provision	Methodology	Principle	Type
Saxitoxin Group	AOAC Official Method 2005.06 (Paralytic Shellfish Poisoning Toxins in Shellfish) four matrices and 12 toxins	LC-FL	II

I-9 DEFINITION OF DEFECTIVES

A sample unit shall be considered as defective when it exhibits any of the properties defined below.

I-9.1 Foreign Matter

The presence in the sample unit of any matter which has not been derived from bivalve molluscs, does not pose a threat to human health and is readily recognized without magnification or is present at a level determined by any method including magnification, that indicates non-compliance with good manufacturing and sanitation practices.

I-9.2 Dead or Damaged Product

The presence of dead or damaged product. Dead product is characterised by no response to percussion (i.e. shellfish will close by themselves when tapped). Damaged product includes product that is damaged to the extent that it can no longer function biologically. A sample unit shall be considered defective if dead or damaged bivalve molluscs exceed 5% by count.

I-10 LOT ACCEPTANCE

A lot shall be considered as meeting the requirements of this standard when:

(i) the total number of defectives as classified according to section I-8 does not exceed the acceptance number (c) of the appropriate sampling plan in the General Guidelines on Sampling (CAC/GL 50-2004);
(ii) the total number of sample units not meeting the count designation as defined in section I-7.3 does not exceed the acceptance number (c) of the appropriate sampling plan in the General Guidelines on Sampling (CAC/GL 50-2004);
(iii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any individual container;
(iv) the Food Additives, Contaminants, Hygiene and Labelling requirements of Sections I-4, I-5, I-6 and I-7 are met.

PART II – RAW BIVALVE MOLLUSCS

II-2 DESCRIPTION

II-2.1 Product Definition

Raw bivalve molluscs processed for direct consumption or for further processing are products that were alive immediately prior to the commencement of processing and comply with Section I-2.2 relating to harvesting, purification and relaying. They have been shucked and/or frozen and/or processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs. Raw bivalve molluscs are marketed in a frozen or chilled state.

II-2.2 Process Definition

Raw bivalve molluscs must meet the process definition in I-2.2 before they can be processed for direct consumption or further processing.

Bivalve molluscs that have been processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs are ones that have been processed to assure reduction or limitation of the target organisms to the satisfaction of the official agency having jurisdiction.

II-2.3 Presentation

Any presentation of the product shall be permitted provided that it:

- meets all requirements of this standard; and
- is adequately described on the label to avoid confusing or misleading the consumer.

The bivalve molluscs may be packed by weight, count, count per unit of weight, volume or per package.

II-3 ESSENTIAL COMPOSITION AND QUALITY FACTORS

II-3.1 Raw Bivalve Molluscs

Raw bivalve molluscs shall be of a quality fit for human consumption.

II-3.2 Other Ingredients

The packing medium and all other ingredients used shall be of food grade quality and conform to all applicable Codex standards.

II-3.3 Final Product

Raw bivalve molluscs shall meet the requirements of this standard when lots examined in accordance with Section II-9 comply with the provisions set out in Section II-8. Raw bivalve molluscs shall be examined by the methods given in Section II-7.

II-4 FOOD ADDITIVES

Only the use of the following additives is permitted in raw bivalve molluscs.

Antioxidants

For chilled shucked molluscs any antioxidant listed in food category 09.1.2 (Fresh Molluscs, crustaceans and echinoderms) of the General Standard for Food Additives (CODEX STAN 192-1995).

For raw frozen molluscs any antioxidant listed in food category 09.2.1 (Frozen fish, fish fillets, and fish products, including molluscs, crustaceans, and echinoderms) of the General Standard for Food Additives (CODEX STAN 192-1995).

II-5 CONTAMINANTS

Raw bivalve molluscs should meet the requirements of I-5.

II-6 HYGIENE AND HANDLING

II-6.1 It is recommended that the products covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1-1969), the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003).

I-6.2 The products should comply with any microbiological criteria established in accordance with the Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1997).

II-6.3 Bivalve molluscs should meet the requirements of I-6.3 and I-5.4. They should retain visual characteristics associated with freshness, including, where relevant, shells free of dirt.

II-7 LABELLING

In addition to the provisions of the Codex General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985) the following specific provisions apply:

II-7.1 The Name of the Food

The name of the food to be declared on the label shall be the common or usual name of the species of bivalve molluscs in accordance with the law and custom of the country in which the food is sold and in a manner not to mislead the consumer.

II-7.1.1 There shall appear on the label, reference to the presentation provided for in Section II-2.3-Presentation in close proximity to the name of the product in such descriptive terms that will adequately and fully describe the nature of the presentation of the product to avoid misleading or confusing the consumer.

II-7.1.2 In addition to the specified labelling designations above, the usual or common trade names of the variety may be added so long as it is not misleading to the consumer in the country in which the product will be distributed.

II-7.2 Content Declaration

Raw bivalve molluscs shall be labelled by weight, count, count per unit weight, or volume as appropriate to the product.

II-7.3 Storage Instructions
The label shall specify the conditions for storage and/or temperature that will maintain the food safety and characteristics of the product during transportation, storage and distribution including date of minimum durability and date of shucking.

II-7.4 Labelling of Non-retail Containers
Refer to I-6.4 Labelling of Non-retail Containers.

II-7.4.1 Every package containing bivalve molluscs that have been processed to reduce or limit target organisms must be provided with a label certifying that all molluscs have been processed to reduce the target organism to levels acceptable to the official agency having jurisdiction.

II-7.4.2 Safety claims for bivalve molluscs processed to reduce or limit target organisms should be specific to the target organisms that have been reduced or limited as described in the Code of Practice.

II-8 SAMPLING, EXAMINATION AND ANALYSES
II-8.1 Sampling
Sampling of lots for examination of net weight shall be carried out in accordance with an appropriate sampling plan meeting the criteria established by the CAC.

II-8.2 Sensory and Physical Examination
Samples taken for sensory and physical examination shall be assessed by persons trained in such examination and in accordance with procedures elaborated in Sections II-7.3 through II-7.7, and Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories" (CAC/GL 31-1999).

II-8.3 Determination of Net Weight and Drained Weight
The net weight and drained weight of all sample units shall be determined by the procedures described or mentioned in sections II-7.3.1 through II-7.3.5.

II-8.3.1 Determination of Net Weight
(i) Weigh the unopened container;
(ii) Open the container and remove the contents;
(iii) Weigh the empty container, (including the end) after removing excess liquid and adhering meat;
(iv) Subtract the weight of the empty container from the weight of the unopened container.
(v) The resultant figure will be the total net content.

II-8.3.2 Determination of Net Weight of Frozen Products not Covered by Glaze
The net weight (exclusive of packaging material) of each sample unit representing a lot shall be determined in the frozen state.

II-8.3.3 Determination of Net Weight of Products Covered by Glaze
AOAC official method 963.18, Net Contents of Frozen Seafoods

II-8.3.4 The AOAC official method 963.26 should be used to determine the net weight of products with water added that is inside a "block-frozen" product.

II-8.3.5 Determination of Drained Weight
In the case of shucked bivalve molluscs, the drained weight shall be determined according to AOAC official method 953.11.

II-8.4 Determination of Count per Unit Weight or Volume

When declared on the label, the count of bivalve molluscs shall be determined by counting the numbers of bivalve molluscs in the container or a representative sample thereof and dividing the count of bivalve molluscs by the actual weight/volume to determine the count per unit weight or volume.

II-8.5 Sample Preparation

II-8.5.1 Procedures for Thawing

For frozen product, the sample unit is thawed by enclosing it in a film type bag and immersing in water at room temperature (not greater than 35 ºC). The complete thawing of the product is determined by gently squeezing the bag occasionally so as not to damage the texture of the bivalve molluscs, until no hard core or ice crystals are left.

II-8.6 Methods of Analysis of *Escherichia coli*

Refer to I-7.4 Methods of Analysis of *Escherichia coli*.

II-8.7 Determination of Biotoxins

Refer to I-7.5 Determination of Biotoxins

II-9 DEFINITION OF DEFECTIVES

The sample unit shall be considered as defective when it exhibits any of the properties defined below.

II-9.1 Deep Dehydration (Frozen Products)

Greater than 10% of the weight of the bivalve molluscs in the sample unit or greater than 10% of the surface area of the block exhibits excessive loss of moisture clearly shown as white or abnormal colour on the surface which masks the colour of the flesh and penetrates below the surface, and cannot be easily removed by scraping with a knife or other sharp instrument without unduly affecting the appearance of the bivalve molluscs.

II-9.2 Foreign Matter

The presence in the sample unit of any matter which has not been derived from bivalve molluscs, does not pose a threat to human health and is readily recognized without magnification or is present at a level determined by any method including magnification, that indicates non-compliance with good manufacturing and sanitation practices.

II-9.3 Odour/Flavour

Persistent and distinct objectionable odours or flavours indicative of decomposition or rancidity.

II-9.4 Texture

Textural breakdown of the flesh, indicative of decomposition, characterized by muscle structure that is mushy or paste-like.

II-10 LOT ACCEPTANCE

A lot shall be considered as meeting the requirements of this standard when:

(i) the total number of defectives as classified according to section II-8 does not exceed the acceptance number (c) of the appropriate sampling plan in the General Guidelines on Sampling (CAC/GL 50-2004);

(ii) the total number of sample units not meeting the count designation as defined in section II-2.3 does not exceed the acceptance number (c) of the appropriate sampling plan in the General Guidelines on Sampling (CAC/GL 50-2004);

(iii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any individual container;
(iv) the Food Additives, Contaminants, Hygiene and Labelling requirements of Sections II-4, II-5, II-6 and II-7 are met.

Appendix 3

EXAMPLE OF A DEPURATION CYCLE RECORD SHEET

Depuration cycle record sheet

DEPURATION TANK LOADING	Batch number	
	System identifier	
	Tank identifier (for multi-tank systems)	
	Species	
	Source harvesting area	
	Salinity of source area (if known) (ppt)	
	Quantity of shellfish	kg
	Number of trays loaded into tank	

DEPURATION	**Start cycle**	**2–3h Post start**	**Mid cycle**	**End point**
Date	/ /	/ /	/ /	/ /
Time	: hrs	: hrs	: hrs	: hrs
Water level OK	YES NO		YES NO	YES NO
Flow rate l/min				
Salinity (ppt)				
UV lamps OK	YES NO		YES NO	YES NO
UV lamp elapsed usage (hours)				
Water temperature	^{o}C	^{o}C	^{o}C	^{o}C
Water clarity and odour OK	YES NO	YES NO	YES NO	YES NO
DO_2 Entry (spray bar)	YES NO			YES NO
DO_2 Exit (suction bar)	YES NO			YES NO
Mollusc activity OK	YES NO	YES NO	YES NO	YES NO
Initials of operator				
Comments: e.g. record of breakdowns, spawning in tanks, failure of molluscs to function, additions or changing of water, dumping of molluscs, etc.				

Microbiological results for batch

	E. coli or faecal coliforms per 100g		
	Sample 1	Sample 2	Sample 3
Pre-depuration (as received in plant)			
Post-depuration (after drain down)			

Final signature: .....
Date: .....

Appendix 4

US NATIONAL SHELLFISH SANITATION PROGRAMME DEPURATION CRITERIA

United States Food and Drug Administration (2006)

Authors' note: Extracted from the National Shellfish Sanitation Programme: Guide for the Control of Molluscan Shellfish 2005. The full guide contents can be downloaded from the Web site of the Centre for Food Safety and Applied Nutrition of the US Food and Drug Administration (www.cfsan.fda.gov).

II. MODEL ORDINANCE

XV. Depuration

Note: In those States where depuration is not practiced, this Chapter may be deleted from the Ordinance, as well as references to depuration throughout the Ordinance.

REQUIREMENTS FOR THE AUTHORITY

[**Note:** The Authority must meet the requirements of this section even if the Authority does not formally adopt this Chapter in regulation.]

A. Prior to authorizing depuration, the Authority shall develop and maintain an effective program to:
   (1) Control shellstock harvesting by special license in accordance with Chapter VIII. @.01 C.;
   (2) Control shellstock transportation between the harvest area and the depuration facility to prevent shellstock from being illegally diverted to direct marketing;
   (3) Approve the design and construction of the depuration facility or activity including subsequent changes;
B. If shellstock is transported interstate to be depurated, the Authorities in both States shall execute a memorandum of agreement to provide adequate control measures to prevent diversion prior to depuration.
C. The Authority shall review and approve the Depuration Plant Operating Manual prior to granting depuration certification.
D. The Authority shall review the depuration plant performance index and other records as part of the monthly inspections to verify that the process and CCP are effective and the process verification analysis is being performed properly.
E. The Authority shall maintain adequate records for each depuration facility. The following records for each facility shall be kept for the period of five years:
   (1) Inspection reports and reviews of the plant performance in accordance to §D. (above);
   (2) Current Depuration Plant Operation Manuals for each dealer (§.02).
F. The Authority shall assure that each dealer has procedures to assure that no shellstock which has not been depurated is removed from the depuration facility without the direct supervision of the Authority.

REQUIREMENTS FOR THE DEALER

.01 Critical Control Points.

A. Receiving Critical Control Point - Critical Limits. The dealer shall receive and depurate only shellstock which is:

(1) Obtained from a licensed harvester who has:
  (a) Harvested the shellstock from an Approved or Conditionally Approved area in the open status as indicated by the tag; **[C]** and
  (b) Identified the shellstock with a tag on each container or transaction record on each bulk shipment; **[C]** and
(2) Originates from a dealer who has identified the shellstock with a tag on each container or transaction record with each bulk shipment; **[C]** and
(3) Obtained from a special licensed harvester who has:
  (a) Harvested or supervised the harvest of shellstock from a Restricted or Conditionally Restricted area in the open status; **[C]** and
  (b) Identified the shellstock by transaction records which include the harvest area, the special-licensed harvester's name, harvester license number(s), the harvest date, and the amount of shellstock shipped in each lot. **[C]**

B. Processing Critical Control Points - Critical Limits. The dealer shall assure that:
(1) All depuration lots are treated for a minimum of 44 hours; **[C]** and
(2) The water treatment system is operating to design specifications; **[C]** and
(3) All critical limits established during verification of the specific depuration process are being met. **[C]**

C. Finished Shellstock Storage Critical Control Point - Critical Limits. The dealer shall assure that:
(1) If wet storage in artificial bodies of water is practiced, water quality meets the requirements outlined in Chapter X.08; **[C]** and
(2) Once placed under temperature control while in the possession of the dealer, shellstock shall be:
  (a) Iced; **[C]** or
  (b) Placed in a storage area or conveyance maintained at 45° Fahrenheit (7.2° Centigrade) or less; **[C]**and
  (c) Not permitted to remain outside temperature control for more than 2 hours at points of transfer such as loading docks.**[C]**

.02 Sanitation

A. Safety of Water for Processing and Ice Production
(1) Water supply.
  (a) Dealers shall provide a potable water supply in accordance with applicable federal, state and local regulations. **[C]**
  (b) If the water supply is from a private source, the dealer shall make arrangements to have the water supply sampled by persons recognized by the Authority and tested at laboratories sanctioned or certified by the Authority: **[K]**
    (i) Prior to use of the water supply; **[C]**
    (ii) Every six months while the water supply is in use; **[K]** and
    (iii) After any water supply has been repaired and disinfected. **[$S^{C/K}$]**
(2) Ice production. Any ice used in the processing or storage of shucked shellfish shall:
  (a) Be made on-site from potable water in a commercial ice machine; **[C]** or
  (b) Come from a facility approved by the Authority or the appropriate regulatory agency. **[C]**
(3) Shellstock washing
  (a) Water from either a potable water supply, a growing area in the approved classification, a saltwater well approved by the authority, or the restricted area at the time and place of harvest, shall be used to wash shellstock. **[C]**
  (b) If the dealer uses any system to wash shellstock which recirculates water, the dealer shall:
    (i) Obtain approval for the construction or remodeling of the system from the Authority; **[K]**

(ii) Provide a water treatment and disinfection system to treat an adequate quantity of water to a quality acceptable for shellstock washing, which, after disinfection, meets the coliform standards for drinking water; and does not leave any unacceptable residues in the shellstock; **[C]**

(iii) Test wash water daily for bacteriological water quality; **[$S^{C/K}$]**

(iv) Clean, service, and test disinfection units at the frequency necessary to ensure effective disinfection. **[K]**

(c) The dealer may use ultra-violet (UV) disinfection in his recirculating wash water system, provided that the turbidity of the water to be disinfected:

(i) shall not exceed 20 nephelometric turbidity units (NTUs); **[K]** and

(ii) Is measured using the method in the APHA *Standard Methods for the Examination of Water and Wastewater*. **[K]**

(d) Food contact plumbing which is designed and installed to permit effective cleaning and sanitization shall be used. **[C]**

(4) Depuration process water. The dealer shall:

(a) Continuously treat process water with a disinfection system approved by the Authority that does not leave any unacceptable residue in the shellstock; **[C]** and

(b) Verify that the disinfection system produces process seawater with no detectable coliform organisms as measured using an NSSP approved method in the tank influent according to the following sampling protocols.

(i) If the source water is an approved growing area, approved well, or other approved source, then the tank influent produced by each disinfection unit is evaluated once per process batch; **[C]**

(ii) If the source water is a restricted growing area, then:

a. A study meeting the requirements of Chapter X. 08 C.(2)(b) is required; **[C]**

b. The tank influent produced by each disinfection unit is evaluated daily; [C] and

c. Source water prior to final disinfection must meet the water quality criteria for restricted for depuration in accordance with Chapter IV.02. G-H. [C]

(iii) If the source water is a recirculating water system, then:

a. A study meeting the requirements of Chapter X. 08. C.(2) (b) [C] is required; and

b. The tank influent produced by each disinfection unit is verified daily. [C]

c. A prohibited growing area may not be used for source water. [C]

(5) Plumbing and related facilities.

(a) The dealer shall design, install, modify, repair, and maintain all plumbing and plumbing fixtures to:

(i) Prevent contamination of water supplies; **[C]** and

(ii) Prevent any cross-connection between the pressurized potable water supply and water from an unacceptable source. **[C]** The dealer shall install and maintain in good working order devices to protect against backflow and back siphonage. **[K]**

(b) Shellstock storage tanks and related plumbing shall be fabricated from safe materials, and tank construction shall be such that it :

(i) is easily accessible for cleaning and inspection; **[K]**

(ii) is self-draining; **[K]** and

(iii) meets the requirements for food contact surfaces; **[K]** and

(c) Depuration Plant Design and Construction. The dealer shall ensure that:

(i) Depuration tanks, processing containers, and piping are fabricated from non-toxic corrosion-resistant materials and are easily cleanable; **[K]**

(ii) Depuration tank design, hydraulics, and typical container configuration are such that process water is evenly circulated throughout all the shellfish containers within a given tank; **[K]**

(iii) Shellfish containers allow process water to flow freely and uniformity to all shellfish within each container. **[K]**

(6) Depuration unit

(a) Depuration unit including depuration tanks, all reservoir tanks, and related piping shall be fabricated from safe materials, and depuration unit construction is such that it:

(i) Is easily accessible for cleaning and inspection; **[K]**

(ii) Is self-draining; **[K]** and

(iii) Meets the requirements for food contact surfaces. **[K]**

B. Condition and Cleanliness of Food Contact Surfaces.

(1) Equipment and utensil construction for food contact surfaces.

(a) Except for equipment in continuous use and placed in service prior to January 1, 1989, the dealer shall use only equipment which conforms to Shellfish Industry Equipment Construction Guides (August 1993), U.S. Department of Health and Human Services. **[K]**

(b) The dealer shall use only equipment and utensils, including approved plastic ware which is:

(i) Constructed in a manner and with materials that can be cleaned, sanitized, maintained or replaced in a manner to prevent contamination of shellfish products; **[K]**

(ii) Free from any exposed screws, bolts, or rivet heads on food contact surfaces **[K]** and

(iii) Fabricated from food grade materials.**[K]**

(c) The dealer shall assure that all joints on food contact surfaces:

(i) have smooth easily cleanable surfaces; **[K]** and

(ii) are welded. **[K]**

(d) All equipment used to handle ice shall be kept clean and stored in a sanitary manner, and shall meet the construction requirements in §.02 B (1) (a), (b), and (c). **[K]**

(2) Cleaning and sanitizing of food contact surfaces.

(a) Food contact surfaces of the depuration units, equipment and containers shall be cleaned and sanitized to prevent contamination of shellstock and food contact surfaces. The dealer shall:

(i) Provide applicable adequate cleaning supplies and equipment, brushes, detergents, and sanitizers, hot water and pressure hoses. **[K]**

(ii) Wash, rinse and sanitize equipment prior to the start-up of each day's activities and following any interruption during which food contact surfaces may have been contaminated; **[K]**

(b) All conveyances and equipment which come into contact with stored shellstock shall be cleaned and maintained in a manner and a frequency as necessary to prevent shellstock contamination. **[O]**

(c) Containers which may have become contaminated during storage shall be properly washed, rinsed and sanitized prior to use or are discarded. **[K]**

(d) Shellstock depuration tanks shall be cleaned and sanitized on a regular schedule as part of a plant sanitation standard operating procedure. **[K]**

C. Prevention of Cross Contamination.

(1) Protection of shellfish.

(a) Shellstock shall be stored in a manner to protect shellstock from contamination in dry storage and at points of transfer. **[$S^{C/K}$]**

(b) Shellstock shall not be placed in containers with standing water for the purposes of washing shellstock or loosening sediment; **[K]**

(2) Employee practices.
(a) The dealer shall require all employees to wash their hands thoroughly with soap and water and sanitize their hands in an adequate hand washing facility:
(i) Before starting work; **[K]**
(ii) After each absence from the work station; **[K]**
(iii) After each work interruption; **[K]** and
(iv) Any time when their hands may have become soiled or contaminated. **[K]**

D. Maintenance of Hand Washing, Hand Sanitizing and Toilet Facilities
(1) Hand washing facilities with warm water at a minimum temperature of 100° Fahrenheit (38° Centigrade) ,dispensed from a hot and cold mixing or combination faucet, shall be provided; **[$S^{K/O}$]**
(2) Sewage **[C]** and liquid disposable wastes **[K]** shall be properly removed from the facility.
(3) An adequate number of conveniently located toilets shall be provided.**[K]**
(4) The dealer shall provide each toilet facility with an adequate supply of toilet paper **[K]** in a suitable holder. **[$S^{K/O}$]**

E. Protection from Adulterants.
(1) Shellstock shall be protected from contamination while being transferred from one point to another during handling and processing; **[K]**
(2) Any lighting fixtures, light bulbs, skylights, or other glass suspended over food storage or processing activities in areas where shellstock are exposed shall be of the safety type or protected to prevent food contamination in case of breakage. **[O]**
(3) Conveyances or devices used to transport shellstock shall be constructed, maintained and operated to prevent contamination of the shellstock. If overhead monorails or conveyors are used, the dealer shall take precautions to assure that hydraulic fluids or lubricants do not leak or drip onto the shellstock or conveyance surfaces. **[K]**
(4) Adequate ventilation shall be provided to minimize condensation in areas where shellfish are stored, processed or packed. **[$S^{K/C}$]**
(5) Shellstock packing activities shall be conducted to provide adequate protection from contamination and adulteration. **[K]**
(6) Protection of ice used in shellstock shipping.
(a) Any ice which is not made on-site in the depuration facility shall be inspected upon receipt and rejected if the ice is not delivered in a way so as to be protected from contamination. **[$S^{C/K}$]**
(b) Ice shall be stored in a safe and sanitary manner to prevent contamination of the ice. **[$S^{C/K}$]**

F. Proper Labeling, Storage and Use of Toxic Compounds.
(1) Storage of toxic compounds.
(a) The dealer shall assure that only toxic substances necessary for plant activities are present in the facility. **[K]**
(b) Each of the following categories of toxic substances shall be stored separately:
(i) Insecticides and rodenticides; **[K]**
(ii) Detergents, sanitizers, and related cleaning agents; **[K]** and
(iii) Caustic acids, polishes, and other chemicals. **[K]**
(c) The dealer shall not store toxic substances above shellfish or food contact surfaces. **[K]**
(2) Use and labeling of toxic compounds.
(a) When pesticides are used, the dealer shall apply pesticides in accordance with applicable federal and state regulations to control insects and rodents in such a manner to prevent the contamination of any shellfish or packaging materials with residues. **[K]**

(b) Cleaning compounds and sanitizing agents shall be used only in accordance with applicable federal and state laws and regulations. **[K]**
(c) Detergents, sanitizers, and other cleaning supplies shall be used only in strict accordance with the manufacturer's label instructions. **[K]**
(d) Toxic substances shall be used only in strict accordance with the manufacturer's label instructions. **[K]**

G. Control of Employees with Adverse Health Conditions.
(1) The dealer shall take all reasonable precautions to assure that any employee with a disease in the communicable stage which might be transmissible through food shall be excluded from working in any capacity in which the employee may come in contact with the shellfish or with food contact surfaces. The diseases which are transmissible from food workers through food are those determined by the US Centers for Disease Control and Prevention, in compliance with the Americans with Disabilities Act, and published in the *Federal Register.* **[K]**
(2) If an employee with an infected wound keeps it covered with a proper bandage, an impermeable barrier, and a single-use glove for a hand lesion, the dealer may allow the employee to work in the shellfish processing facility without additional restrictions. **[K]**

H. Exclusion of Pests. The dealer shall operate his facility to assure that pests are excluded from his facility and his activities. **[K]**

.03 Other Model Ordinance Requirements

A. Plants and Grounds.
(1) General
(a) The physical facilities shall be maintained in good repair. **[O]**
(b) Animals or unauthorized persons shall not be allowed in those portions of the facilities where shellstock are stored, handled, processed, or packaged and food handling equipment and packaging materials are cleaned or stored. **[K]**
(2) Flooding. Facilities in which shellstock are stored, packed, or repacked shall be located so that these facilities are not subject to flooding during ordinary high tides. If facilities are flooded: **[C]**
(a) Shellstock processing or repacking activities shall be discontinued until the floodwaters have receded from the building; and the building is cleaned and sanitized. **[C]**
(b) Any shellstock coming in contact with the floodwaters while in storage shall be destroyed; or discarded in non-food use. **[C]**
(3) The dealer shall operate his facility to provide adequate protection from contamination and adulteration by assuring that dirt and other filth are excluded from his facility and activities. **[$S^{C/K}$]**
(4) Separation of operations. Manufacturing activities which could result in the contamination of the shellstock shall be separated by adequate barriers. **[K]**
(5) Plant interior.
(a) Sanitary conditions shall be maintained throughout the facility. **[O]**
(b) Interior surfaces are kept in good repair. **[O]**
(c) All dry area floors are hard, smooth, easily cleanable and in good repair; **[O]** and
(d) All wet area floors used in areas to store shellstock, food processing, and cleaning equipment are constructed of easily cleanable, impervious, and corrosion resistant materials which:
(i) Are graded to provide adequate drainage; **[O]**
(ii) Have even surfaces, and are free from cracks that create sanitary problems and interfere with drainage; **[O]** and
(iii) Have sealed junctions between floors and walls to render them impervious to water. **[O]**

(6) Walls and Ceilings. Interior surfaces of rooms where shellstock are stored, handled, processed, or packaged and food handling equipment and packaging materials shall be constructed of easily cleanable, corrosion resistant, impervious and light colored materials. **[O]**

(7) Grounds. Grounds around the facility shall be maintained to be free from conditions which may result in shellfish contamination. These conditions may include:

(a) Rodent attraction and harborage; **[O]**

(b) Inadequate drainage. **[O]**

B. Plumbing and Related Facilities.

(1) Hand washing facilities shall be provided which are:

(a) Convenient to work areas; **[O]**

(b) Separate from the three compartment sinks used for cleaning equipment and utensils **[K]**; and

(c) Directly plumbed to an approved sewage disposal system. **[$S^{O/K}$]**

(2) The dealer shall provide at each hand washing facility:

(a) A supply of hand cleansing soap or detergent; **[K]**

(b) A conveniently located supply of single service towels in a suitable dispenser or a hand drying device that provides heated air; **[O]**

(c) An easily cleanable waste receptacle; **[O]** and

(d) Hand washing signs in a language understood by the employees; **[O]**

(3) All plumbing and plumbing fixtures shall be designed, installed, modified, repaired, and maintained to provide a water system that is adequate in quantity and under pressure, and includes:

(a) Cold and warm water at all sinks; **[K]** and

(b) Hand washing facilities adequate in number and size for the number of employees, and are located where supervisors can observe employee use. **[K]**

(4) Adequate floor drainage, including backflow preventers such as air gaps, shall be provided where floors are:

(a) Used in shellstock storage; **[K]**

(b) Used for food holding units (e.g. refrigeration units); **[K]**

(c) Cleaned by hosing, flooding, or similar methods; **[K]** and

(d) Subject to the discharge of water or other liquid waste, including, if applicable, three compartment sinks, on the floor during normal activities; **[K]**

(5) A safe, effective means of sewage disposal for the facility shall be provided in accordance with applicable federal and state laws and regulations; **[$S^{C/K}$]**

(6) Installation of drainage or waste pipes over processing or storage areas, or over areas in which containers and utensils are washed or stored shall not be permitted. **[K]**

C. Utilities. Ventilation, heating, or cooling systems shall not create conditions that may cause the shellstock to become contaminated. **[$S^{C/K}$]**

D. Insect and Vermin Control. The dealer shall employ necessary internal and external insect and vermin control measures to assure that insects and vermin are not present in the facility, including:

(1) Tight fitting, self-closing doors; **[K]**

(2) Screening of not less that 15 mesh per inch; **[K]** or

(3) Controlled air currents. **[K]**

E. Disposal of Wastes.

(1) Disposal of waste materials shall be conducted in accordance with appropriate federal and state laws and regulations. **[O]**

(2) All areas and receptacles used for the storage or conveyance of waste shall be operated and maintained to prevent attraction, harborage, or breeding places for insects and vermin. **[O]**

F. Equipment Construction for Non-food Contact Surfaces.
   (1) The dealer shall use only equipment which is constructed in a manner and with materials that can be cleaned, sanitized, maintained or replaced in a manner to prevent contamination of shellstock. **[O]**
   (2) The dealer shall use easily cleanable, corrosion resistant, impervious materials, free from cracks, to construct any non-food contact surfaces in shellfish storage or handling areas. **[O]**

G. Cleaning and Sanitizing of Non-food Contact Surfaces.
   (1) Cleaning activities for the depuration unit and equipment shall be conducted in a manner and at a frequency appropriate to prevent contamination of shellstock and food contact surfaces. **[K]**
   (2) All conveyances and equipment which come into contact with stored shellstock shall be cleaned and maintained in a manner and frequency as necessary to prevent shellstock contamination. **[O]**

H. Shellstock Storage and Handling.
   (1) The dealer shall assure that shellstock is:
      (a) Reasonably free of sediment; **[O]** and
      (b) Culled. **[K]**
   (2) Shellstock shall be stored in a protected location which assures complete and rapid drainage of water away from the shellstock by:
      (a) Placing shellstock at an adequate height off the floor; **[K]**or
      (b) Grading the floor. **[O]**
   (3) Any mechanical refrigeration equipment used for shellstock storage shall be adequate in size and are equipped with:
      (a) An automatic temperature regulating control; **[K]** and
      (b) Installed thermometers to accurately measure temperature within the storage compartments. **[K]**
   (4) Inspect incoming shipments and shall reject dead or inadequately protected shellstock. **[K]**
   (5) Ensure that separate dry storage facilities are provided for depurated and undepurated shellfish. **[K]**
   (6) Cull and wash the shellstock prior to loading into the depuration tanks. This process may occur before the shellstock is received at the facility by;
      (a) Licensed harvester(s) at the harvest site; **[K]** or
      (b) Certified dealer(s) at their certified facility. **[K]**
   (7) Assure that culled shellfish are destroyed or disposed of in such a manner as to prevent their use for human food. **[K]**
   (8) Transport, store, and handle shellstock so that:
      (a) Shellstock potential for normal physiological activity during depuration is not compromised; **[K]** and
      (b) Shellstock quality is not degraded. **[K]**
   (9) Assure that different harvest lots of shellfish are not commingled during washing, culling, processing, or packing. If more than one harvest lot of shellfish is being processed at the same time, the identity of each harvest lot is maintained throughout the stages of depuration. **[K]**
   (10) Wash and cull shellstock after depuration and pack the shellstock in clean shipping containers fabricated from safe materials. **[K]**
   (11) Depurated packaged shellstock shall be protected from contamination at all times and be held at an ambient temperature not to exceed 45° Fahrenheit (7.2° Centigrade). **[K]**

I. Heat Shock. N/A

J. Personnel. Any employee handling shucked shellfish shall be required to:
   (1) Wear effective hair restraints; **[O]**
   (2) Remove any hand jewelry that cannot be sanitized or secured; **[O]**

(3) Wear finger cots or gloves if jewelry cannot be removed; **[O]**
(4) Wear clean outer garments, which are rinsed or changed as necessary to be kept clean. **[O]**
(5) In any area where shellfish are shucked or packed and in any area which is used for the cleaning or storage of utensils, the dealer shall not allow employees to:
(a) Store clothing or other personal belongs; **[O]**
(b) Eat or drink; **[K]**
(c) Spit; and **[K]**
(d) Use tobacco in any form. **[K]**

K. Supervision.
(1) A reliable, competent individual shall be designated to supervise general plant management and activities; **[K]**
(2) Cleaning procedures shall be developed and supervised to assure cleaning activities do not result in contamination of shellstock or food contact surfaces. **[K]**
(3) All supervisors shall be:
(a) Trained in proper food handling techniques and food protection principles; **[K]** and
(b) Knowledgeable of personal hygiene and sanitary practices. **[K]**
(4) The dealer shall require:
(a) Supervisors to assure that proper sanitary practices are implemented, including:
(i) Plant equipment clean up; **[K]**
(ii) Rapid product handling; **[K]** and
(iii) Shellstock protection from contamination. **[K]**
(b) Employees
(i) to be trained in proper food handling and personal hygiene practices, **[K]** and
(ii) to report any symptoms of illness to their supervisor. **[K]**

L. Plant Operating Manual. The dealer shall prepare a written Depuration Plant Operations Manual (DPOM) according to Minimum Requirements of a Depuration Plant Operations Manual (below); and update the DPOM as necessary. A copy of the DPOM shall be kept in a location readily accessible to the trained personnel responsible for the depuration activity. The minimum requirements for a Depuration Plant Operating Manual shall address:
(1) Introduction including;
(a) Status of document (to create, revise, or update DPOM);
(b) Ownership and principal(s) involved with operation of facility;
(c) Address and phone number of owners and principles; and
(d) Summary of proposed use of the depuration facility including statement of objectives of the operation of the plant, species to be processed, proposed periods of facility operation, proposed sources of shellfish, including potential harvest areas, and maximum capacity of plant.
(2) Description of the Facility including;
(a) Site plan drawings;
(b) Facility layout including detailed schematic of the entire depuration system;
(c) Schematic drawing of process;
(d) Product flow diagram showing product movement through facility (may be combined with §B.(3);
(e) Statement that construction materials and fabrication will meet the requirements of §.04, §.08, and §.09; and
(f) Schematic of seawater delivery and distribution system.
(3) Design Specifications of Depuration Unit including;
(a) Depuration tank diagram including tank dimensions and construction details, influent and effluent locations, operating water level, and typical container configuration;

(b) Process water system describing type of system (flow-through or recirculating), pretreatment and filtration systems, disinfection system, and hydraulic schematic;
(c) Shellfish containers construction and material meets §.04 and §.08 of this Chapter; and
(d) List of equipment including washing, culling, and packing equipment, material handling equipment, and cleaning and sanitation equipment.

(4) Laboratory to be utilized for microbial analyses (in house, government agency, private commercial);
(5) Depuration process monitoring including:
(a) Sampling protocols including frequency of sampling, number of samples, sampling locations, and methodology for process water analyzing, incoming shellstock, depurated shellstock, and growing waters;
(b) Monitoring equipment maintenance and calibration procedures and copy of activity log forms that will be used for data entry;
(c) Process water monitoring protocol for physical and chemical parameters; and
(d) Data analysis and evaluation.

(6) Standard Operating Procedure for:
(a) Receiving and holding;
(b) Washing, culling, and placement of undepurated product in process tanks;
(c) Depuration unit operation;
(d) Monitoring of depuration unit operation;
(e) Removal of depurated product from process tanks;
(f) Storage parameters and procedures;
(g) Labeling/tagging procedures;
(h) Plant cleaning and sanitation; and
(i) Data analysis.
(j) Recall procedures.

(7) Record Keeping. List categories of information that will be recorded. Include copies of proposed forms to be used in each category. A single form may be used for several categories if properly designed.
(a) Shipping and receiving records;
(b) Plant Operation Log, including provisions for recording the values for chemical and physical parameters;
(c) Maintenance and Sanitation Log(s);
(d) Laboratory records;

M. Process Verification. The Dealer shall continually:
(1) Perform process verification on a continuous basis according to the following protocol:
(a) Following completion of a minimum of 44 hours of depuration, collect and assay at least one end-product sample from each lot of shellstock to be depurated in the depuration unit.
(b) Determine daily, or as results become available, the depuration performance indices defined as the geometric mean and 90^{th} percentile of fecal coliform (FC) from assay data of the most recent ten (10) consecutive harvest lots for each species depurated and for each restricted harvest area used.
(c) Compare daily, or as a results become available, the depuration performance indices with the following Critical Limits for the Indices of Depuration Plant Performance.
(d) If the depuration performance indices for a specific species from a specific growing area are less than or equal to the above Critical Limits for the Indices of Depuration Plant Performance, then the process is considered verified for that species from that growing area.

Limits for verification of depuration plant performance fecal Coliform per 100 grams		
Species	**Geometric mean**	**90th Percentile**
Soft Clams (*Mya arenaria*)	50	130
Hard Clams (*Mercenaria mercenaria*)	20	70
Oysters	20	70
Manial clams	20	70
Mussels	20	70

(e) For the purpose of making calculations, fecal coliform counts that signify the upper or lower limit of sensitivity of the test (MPN or ETCP) shall be increased or decreased by one significant figure. Thus, <9.0 becomes 8.9, <17 becomes 16 and >248 becomes 250. Individual plates which are too numerous to count (TNTC) are considered to have >100 colonies per plate. A sample containing "TNTC" plates is collectively rendered as having a count of 10 000.

(2) Conditional Protocol Verification. If the depuration performance indices for a specific growing area fail to meet the Critical Limits for the Indices of Depuration Plant Performance, or if a new restricted growing area is used as a source of shellfish for depuration, or if a new depuration process has generated less than 10 process batches of data, the process is considered to be unverified and the dealer shall adhere to the following conditional protocols:

(a) The depuration processor shall collect and assay at least one zero hour and three end-product samples from each harvest lot;

(b) Environmental parameters including process water temperature, salinity, dissolved oxygen, and turbidity and/or other operational conditions may inhibit the physiological process and must be identified. The conditions(s), once identified and quantified, become critical control points (CCP) for specific species in the specific plant and the hazard analysis and HACCP plan shall be revised accordingly;

(c) Shellstock which are processed during this conditional protocol must meet the following release criteria before they may be released to market:

(i) Geometric mean (from three samples) of soft clams not to exceed 110 and no single sample to exceed 170; or

(ii) Geometric mean (from three samples) of other clam species, mussels, or oysters not to exceed 45 and no single sample to exceed 100.

(d) If the harvest lot fails to meet the release criteria, the depuration processor may choose to subject the product to additional depuration processing whereupon the shellfish can be resampled for release criteria or the disposition of the shellfish shall be as follows:

(i) The Authority, in consultation with the depuration processor, may order the destruction of the shellfish; or

(ii) The Authority, in consultation with the depuration processor, may allow non-food use of the shellfish; or

(iii) The Authority, in consultation with the depuration processor, may allow the shellfish to be relayed in accordance with Chapter V.

(e) When in Conditional Protocol Verification due to a failure of an established harvest area to meet the above Indices for Depuration Plant Performance, determine daily, or as results become available, the depuration performance indices defined as the geometric mean and 90th percentile of fecal coliform (FC) from assay data of the most recent ten (10) consecutive end product samples for each species depurated and for each harvest area used

(i) Compare these depuration performance indices with the above Critical Limits for the Indices of Depuration Plant Performance for this species.

(ii) If these depuration performance indices are less than or equal to the above Critical Limits for the Indices of Depuration Plant Performance for this species, the process is then considered to be verified for this species from this particular harvest area; and the process reverts to the Process Verification protocol in .03L (1) .

(iii) If either the geometric mean or the 90th percentile values exceed the above Critical Limits for the Indices of Depuration Plant Performance for this species, the process shall remain in Conditional Protocol Verification for this species from this particular harvest area until the above Indices of Depuration Plant Performance are attained.

(f) When in Conditional Protocol Verification due to the use of a new harvest area as the source of shellfish or if a new depuration process has generated less than 10 process batches of data, determine daily, or as results become available, the depuration performance indices defined as the geometric mean and 90th percentile of fecal coliform (FC) from assay data of the most recent ten (10) consecutive harvest lots for each species depurated and for each harvest area used.

(i) Compare these depuration performance indices with the above Critical Limits for the Indices of Depuration Plant Performance for this species.

(ii) If these depuration performance indices are less than or equal to the above Critical Limits for the Indices of Depuration Plant Performance for this species, the process is then considered to be verified for this species from this particular harvest area; and the process reverts to the Process Verification protocol in XV. 03 L . (1).

(iii) If less than 10 process batches of data have been collected or either the geometric mean or the 90th percentile values exceed the above Critical Limits for the Indices of Depuration Plant Performance for this species, from this particular harvest area, the process shall remain in Conditional Protocol Verification for this species from this particular harvest area until 10 batches of data have been collected and the above Indices of Depuration Plant Performance are attained.

(3) When depuration units with multiple tanks are used, it is necessary to determine whether the individual tanks are similar.

(a) Tanks are considered similar if the difference between physical tank dimensions and process water flow rate is less than 10%.

(b) If they are not similar, then the process verification protocols contained in Section .03 (1) - (2) must be employed for each tank.

(4) The dealer shall ensure that all microbiological assays of end-point samples of shellstock:

(a) Are analyzed by a laboratory which has been evaluated and approved pursuant to the requirements in Chapter III, using an NSSP-approved method;

(b) Sample size consists of a pool of at least 12 shellfish selected at random from each designated container (more than 12 individuals may be required in the case of smaller shellfish); and

(c) Samples are collected at locations within the depuration unit that are considered to be most compromised as regards shellfish activity, based on the sampling plan contained in the Depuration Plant Operations Manual.

Appendix 5

WHO GUIDELINES ON DRINKING WATER QUALITY

Summary tables of recommendations on chemical quality and microbial verification

Authors' note: The tables given here are taken from the WHO Guidelines for Drinking-water Quality which explains the requirements to ensure drinking-water safety, including minimum procedures and specific guideline values, and how those requirements are intended to be used. The volume also describes the approaches used in deriving the guidelines, including guideline values. It includes fact sheets on significant microbial and chemical hazards.

The tables contain guideline maximum levels for a range of chemical contaminants and faecal bacterial indicators. Unless local regulations stipulate different maximum levels, these recommendations may be used to determine the suitability of water for use in depuration plants, including the preparation of artificial seawater.

The Guidelines themselves can be downloaded from the Web site of the World Health Organization (www.who.int).

Table 7.7: Guideline values for verification of microbial quality[a]

Organisms	Guideline value
All water directly intended for drinking	
E. coli or thermotolerant coliform bacteria[b,c]	Must not be detectable in any 100 ml sample
Treated water entering the distribution system	
E. coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100 ml sample
Treated water in the distribution system	
E coli or therrnotolerant coliforrn bacteria[b]	Must not be detectable in any 100 ml sample

[a] Immediate investigative action must be taken if *E. coli* are detected.

[b] Although *E. coli* is the more precise indicator at faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of water supplies, particularly in tropical areas, where many bacteria of no significance occur in almost all untreated supplies.

[c] It is recognized that in the great majority of rural water supplies, especially in developing countries, faecal contamination is widespread. Especially under these conditions, medium-term targets for the progressive improvement of water supplies should be set.

Table 8.18: Guideline values for naturally occurring chemicals that are of health significance in drinking-water

Chemical	Guideline value[a] (mg/litre)	Remarks
Arsenic	0.01 (P)	–
Barium	0.7	–
Boron	0.5 (T)	–
Chromium	0.05 (P)	For total chromium
Fluoride	1.5	Volume of water consumed and intake from other sources should be considered when setting national standards
Manganese	0.4 (C)	–
Molybdenum	0.07	–
Selenium	0.01	–
Uranium	0.015 (P,T)	Only chemical aspects of uranium addressed

[a] P = provisional guideline value, as there is evidence of a hazard, but the available information on health effects is limited; T= provisional guideline value because calculated guideline value is below the level that can be achieved through practical treatment methods, source protection etc.; C = concentrations of the substance at or below the health-based guideline value may affect the appearance, taste or odour of the water, resulting in consumer complaints.

Table 8.21: Guideline values for chemicals from industrial sources and human dwellings that are of health significance in drinking-water

Inorganics	Guideline value[a] (mg/litre)	Remarks
Cadmium	0.003	–
Cyanide	0.07	–
Mercury	0.001	For total mercury (inorganic plus organic)
Organics	**Guideline value[a] (µg/litre)**	**Remarks**
Benzene	10[b]	–
Carbon tetrachloride	4	–
Di(2-ethylhexyl)phthalate	8	–
Dichlorobenzene, 1,2-	1 000 (C)	–
Dichlorobenzene, 1,4-	300 (C)	–
Dichloroethane, 1,2-	30[b]	–
Dichloroethene, 1,2-	50	–
Dichloromethane	20	–
Dioxane, 1,4-	50[b]	
Edetic Acid (EDTA)	600	Applies to the free acid
Ethylbenzene	300 (C)	–
Hexachlorobutadiene	0.6	–
Nitrilotriacetic Acid (NTA)	200	–
Pentachlorophenol	9[b] (P)	–
Styrene	20 (C)	–
Tetrachloroethene	40	–
Toluene	700 (C)	–
Trichloroethene	20 (P)	–
Xylenes	500 (C)	–

[a] P = provisional guideline value, as there is evidence of a hazard, but the available information on health effects is limited; C = concentrations of the substance at or below the health-based guideline value may affect the appearance, taste or odour of the water, resulting in consumer complaints.

[b] For non-threshold substances, the guideline value is the concentration in drinking-water associated with an upper-bound excess lifetime cancer risk of 10^{-5} (one additional cancer per 100 000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years). Concentrations associated with estimated upper-bound excess lifetime cancer risks of 10^{-4} and 10^{-6} can be calculated multiplying and dividing, respectively, the guideline value by 10.

Table 8.24: Guideline values for chemicals from agricultural activities that are of health significance in drinking-water

Non-pesticides	**Guideline value[a] (mg/litre)**	**Remarks**
Nitrate (as NO_3^-)	50	Short-term exposure
Nitrite (as NO_2^-)	3	Short-term exposure
	0.2 (P)	Long-term exposure
Pesticides used in agriculture	**Guideline value[a] (µg/litre)**	**Remarks**
Alachlor	20[a]	–
Aldicarb	10	Applies to aldicarb sulfoxide and aldicarb sulfone
Aldrin and dieldrin	0.03	For combined aldrin plus dieldrin
Atrazine	2	–
Carbofuran	7	–
Chlordane	0.2	–
Chlorotoluron	30	–
Cyanazine	0.6	–
2,4-D (2,4-dichlorophenoxyacetic acid)	30	Applies to the free acid
2,4-DB	90	–
1,2-Dibromo-3-chloroprepane	1[b]	–
1,2-Dibromoethane	0.4[b] (P)	–
1,2-Dichloropropane (1,2-DCP)	40 (P)	–
1,3-Dichloroprepane	20[b]	–
Dichlorprop	100	–
Dimethoate	6	–
Endrin	0.6	–
Fenoprop	9	–
Isoproturon	9	–
Lindane	2	–
MCPA	2	–
Mecoprop	10	–
Methoxychlor	20	–
Metolachlor	10	–
Molinate	6	–
Pendimethalin	20	–
Simazine	2	–
2,4,5-T	9	–
Terbuthylazine	7	–
Trifluralin	20	–

[a] P = provisional guideline value, as there is evidence of a hazard, but the available information on health effects is limited.

[b] For substances, that are considered to be carcinogenic, the guideline value is the concentartion in drinking-water associated with an upper-bound excess lifetime cancer risk of 10^{-5} (one additional cancer per 100 000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years). Concentrations associated with estimated upper-bound excess lifetime cancer risks of 10^{-4} and 10^{-6} can be calculated multiplying and dividing, respectively, the guideline value by 10.

Appendix 6

LOBSTER STORAGE AND SHELLFISH PURIFICATION

NOTES ON THE SALINITY OF SEAWATER AND
THE USE OF ARTIFICIAL SEAWATER IN
COMMERCIAL INSTALLATIONS

Laboratory Leaflet (New series) **No. 13**

FISHERIES LABORATORY
MINISTRY OF AGRICULTURE, FISHERIES AND FOOD
BURNHAM ON CROUCH, ESSEX

AUGUST 1966

Authors' note: Although the leaflet reproduced here is old it contains the fullest readily available information on the preparation of artificial seawater for the depuration of a number of important species of shellfish.

CONTENTS

Laboratory Leaflet (New Series) No. 13

LOBSTER STORAGE AND SHELLFISH PURIFICATION

Notes on the salinity of seawater and the use of artificial seawater in commercial installations

INTRODUCTION

Within recent years there has been a steady increase in the number of shore installations where lobsters are stored or oysters are purified. The water used in these tanks is usually pumped from the sea, but in some cases artificial seawater is made up from a mixture of simple salts. Where water is taken from an estuary there is a risk that the salinity may at times be too low to permit the normal activities of the shellfish. The object of this leaflet is to describe how the salt content of sea-water can be measured, and how salts may be used to increase the salinity of natural seawater, or for the manufacture of artificial seawater for use in lobster storage and shellfish purification tanks.

1. WHAT IS SALINITY AND HOW DOES IT VARY?

The salt content or salinity of seawater is usually expressed as the number of parts by weight of salt in one thousand parts by weight of water. The unit "parts per thousand" is usually indicated by the symbol ‰. Thus water having a salinity of 35‰ contains 35 lb of salt in 100 gallons. For those wishing to use metric units, water of salinity 35‰ contains 35 g of salt in 1 litre of water, or 35 kg in 1 cubic metre (m^3).

The salinity of seawater usually decreases as one move from the open sea into an estuary, as a result of the increased quantity of fresh water present. In the open sea around the British Isles salinities of 34‰ or more as usual, with only small changes during the seasons. However, in tidal estuaries salinities are generally lower and subject to considerable variation. Salinities are usually lower in winter than in spring tides. At the seaward end of a typical east coast oyster-producing estuary the maximum range of salinity during the year may be found from 26–34‰, whilst at the upper limit of oyster cultivation the salinity in winter may vary from 10–25‰ during a tidal cycle. In addition to these changes, local areas of low salinity may be found close inshore adjacent to freshwater discharges from streams or outfall pipes. Also, water near the surface may be of considerably lower salinity than that found at deeper levels, for there is a tendency for fresh water, or seawater containing a large proportion of fresh water, to remain on the surface. For this reason, intakes to seawater installations should be placed on or near the bottom in as deep a water as possible.

2. THE MEASUREMENT OF SALINITY

It is difficult to measure the salt content of seawater by direct means, but a good estimate of the water quality can be obtained by measuring its specific gravity with a hydrometer. For rough work, only the specific gravity need be considered, but for a more accurate estimate the temperature of the water must also be taken so that salinity can be obtained by reference to a table or a graph. Distilled water has a specific gravity of about 1.000 and "full" seawater of about 1.026, but these values vary a little according to the water temperature. It is important to distinguish clearly between salinity and specific gravity when describing seawater, for the specific gravity is often referred to by the last two numbers only. For the tank operator there are a number

of hydrometers available for the measurement of specific gravity, but one which is particularly useful is listed as: – Soil testing hydrometer, long stem, to BS 1377, range 0.995–1.030 SG at 20 °C. If other instruments are used, care should be taken to ensure that the graduations are sufficiently wide apart to permit accurate reading, and that the instrument, if used with the tables and graph appended to this leaflet, is calibrated between 17.5 and 20 °C. When ordering a hydrometer, it is advisable to ask for a glass hydrometer jar of suitable size to go with it.

To determine the specific gravity, a sample of water should be taken from the tanks or from the incoming seawater in a clean vessel, free from oil or grease. The bulb and stem of the hydrometer should be cleaned and freed from adhering particles, salt crystals, pieces of cotton wool, grease, etc., and immersed in water in the hydrometer jar. Only the very top of the stem should be handled, for grease from the hand may affect readings. Any bubbles of air seen on the side of the hydrometer bulb should be removed by gentle agitation of the instrument, or by wiping with a clean cloth. The hydrometer taken with the eye level with the water surface. This is why it is important to place the hydrometer in a glass jar when the reading is taken; accurate readings cannot be made in a tank where the hydrometer is viewed from above. The readings shown on the hydrometer is viewed from above. The readings shown on the hydrometer are for specific gravity but only the last two numbers are shown, i.e. 1.020 is usually market as "20"on the scale.

3. SALINITY REQUIREMENTS FOR SHELLFISH TANKS

Lobsters are typically coastal animals found in waters having a salinity of 33‰ or more. They cannot tolerate low salinities, or rapid changes of salinity, and do not occur in large numbers in estuaries or other areas subject to low salinities. It is possible to store lobsters in water having a salinity down to 25‰, and even less when water temperatures are below 50 °F (10 °C), but the minimum value usually considered acceptable in commercial storage units is 27‰. Lobsters exposed to low salinity may weaken and die, with a characteristic swelling in the middle of the body, between the head and the tail region.

Native and Portuguese oysters and hard clams are typically estuarine shellfish which can tolerate relatively low and rapid changes of the salinity. Although these shellfish may become gradually adjusted to the very low salinities which often result from the increasing quantities of fresh water entering an estuary in autumn and winter, the minimum salinity normally considered acceptable in purification plants is 25‰ for native oysters, 20.5‰ for Portuguese oysters and 20‰ for hard clams. In comparison, the minimum salinity for mussel purification is 19‰. Shellfish held in water of too low a salinity will not open, and purification cannot take place; prolonged exposure to low salinity may ultimately lead to death.

For normal purposes a measurement of specific gravity is adequate for ensuring that water has a salinity equal to or greater than the minimum values shown above. The minimum specific gravities of seawater recommended are as follows:

Shellfish	Minimum specific gravity
For storage	
Lobsters	1.023
For purification	
Native oysters	1.022
Portuguese oysters	1.018
Hard clams	1.017
Mussels	1.016

Seawater at any temperature having a specific gravity equal to or greater than the values shown is suitable for use in tanks for the purpose indicated.

If water taken into a tank has a specific gravity near to or below that recommended (say 1.021 for native oysters) it is well worth making a more accurate estimate of the salt content by taking the water temperature and converting the values to salinity. This can be done by reference to the graph enclosed within this leaflet. Starting at the observed temperature, move the finger vertically until it reaches the line for observed specific gravity. At this point move the finger horizontally to either side of the graph, until it cuts the scale where the salinity is shown. Thus water having an SG of 1.020 at 41 °F (5 °C) indicates a salinity of 24‰, which is suitable for the purification of portuguese oysters, clams and mussels, but not Native oysters, nor for the storage of lobsters. The minimum salinities normally accepted in tanks holing the various shellfish are shown on the graph by the thick horizontal lines. If the observed salinity is below the minimum, then a salt mixture as described later should be added. For those not wishing to use the graph, Table 1 has been prepared, showing the minimum specific gravity of seawater at several temperature ranges in various types of installation. It can be seen from the table that as the water temperature rises, the minimum acceptable specific gravity falls below that given in the rough guide. Thus when the specific gravity is less than that recommended in the rough guide, and particularly where large volumes of water are involved, the accurate measurement of salinity using a temperature correction may indicate that water of adequate salinity is present, and so save the additional cost and time involved in adding salts.

In this leaflet, detailed attention is given only to those British species stored or purified commercially, although within recent year there has been increased interest in the live storage of other shellfish.[1] The American lobster (*Homarus americanus*) is known to tolerate salinities suitable for the storage of British lobsters. The crawfish (*Palinurus vulgaris*), otherwise known as the spiny lobster or langouste, is stored in tanks in the south-west of England, where salinities are relatively high, and being an offshore animal is probably intolerant of very low salinities. Recent experiments at the Burnham laboratory indicated that a salinity of 28‰ was too low, whilst 32‰ (approximately SG 1.025–26) was satisfactory. The Norway lobster (*Nephrops norvegicus*), known as Dublin Bay prawn, langoustine, or scampi, is and offshore animal, and in the absence of more detailed information it is recommended that water for its storage should have a salinity of at least 34‰ (approximately SG 1.027–28). When artificial seawater is used the weight of salts should be increased, above that shown for lobsters in Table 3, by approximately 7 per cent for crawfish and 13 per cent for Norway lobsters. The edible crab (*Cancer pagurus*)should be held in water containing at least 30‰ of salt (SG 1.024–1.025).

Table 1: Minimum specific gravity of water for use in shellfish installations

Water temperature		Storage of lobsters	Purification of			
°F	°C		Native oysters	Portuguese oysters	Hard clams	Mussels
Up to 50	Up to 10	1.023	1.022	1.018	1.017	1.016
51–59	10.1–15	1.022	1.021	1.017	1.017	1.016
60-68	15.1–20	1.021	1.020	1.016	1.016	1.015
69 and above	20.1 and above	1.020	1.019	1.015	1.015	1.014

[1] The Latin names of the species of shellfish at present stored or purified commercially in this country are as follows: Lobster (*Homarus vulgaris*); Native oyster (*Ostrea edulis*); Portugues oyster (*Crassostrea angulata*); mussel (*Mytilus edulis*); hard clam (*Venus mercenaria*).

Of the remaining commercial species of shellfish, winkles (*Littorina littorea*) are often stored in seawater prior to dispatch to market. These shellfish are estuarine animals able to tolerate a wide range of salinities, at least down to 20‰ (approximately SG 1.016–17), and probably lower. Escallops (*Pecten maximus*), although not normally stored commercially, can be held in tanks of seawater of good salinity. In the absence of any more precise information it is recommended that escallops should not be held in water of salinity less than about 34‰ (approximately SG 1.027–28).

4. THE USE OF SALTS FOR MAKING ARTIFICIAL SEAWATER

Seawater consists of a complex mixture of salts, many of which are present in very small quantities, but for lobster storage and shellfish purification water containing a mixture of five simple salts is adequate. The mixture recommended in this leaflet was devised by Dr Wilder in Canada for the storage of lobsters and has been successfully used in Britain in several commercial storage units. The salt mixture may be used for making up artificial seawater. Water for use in lobster storage and shellfish purification plants contains the same basic mixture of salts, but, for shellfish purification, lower concentrations are employed in order to reduce cost. When more than one type of shellfish is present in an installation the water should be suitable for the shellfish requiring the highest salinity.

The quantities of each of the five salts required for making up amounts of between 50 and 1 000 lb of the salt mixture are shown in Table 2. In Table 3 are shown the individual weights of each salt and the weights of the salt mixture required for making up between 50 and 1 000 gallons[2] of artificial seawater suitable for lobsters, Native oysters, and Portuguese oysters and hard clams respectively. At the time of writing it has not been found economic to make up artificial seawater for the purification of mussels, although there is no practical reason why this should not be done.

The cost of making up artificial seawater may vary widely, depending on the supplier, the area of purchase and the quantity of each salt purchased. Commercial or agricultural grades, obtained through industrial chemists, are suitable and are usually much cheaper than salts to BP (British Pharmacopeia) or analytical reagent quality, which are unnecessary and too expensive. It is therefore well worth making a number of enquiries before buying. One hundredweight lots are always considerably cheaper than smaller quantities. The minor salts are obtainable in quantities of less than one hundredweight, but at considerably higher prices. If salts are bought in quantity and stored before use, airtight containers of plastic or metal should be used, to prevent absorption of water; the salts may be mixed together and stored until required.

The costs of making up artificial seawater with salts purchased in the London area, based on the highest and lowest quotation, are as follows:

Water at recommended salinity	Cost per 100 gallons at 1966 prices
Lobster storage	6s. 9d.–23s. 6d.
Purification of: – Native oysters	6s. 1d.–21s. 2d.
– Portuguese oysters & hard clams	5s. 0d.–17s. 4d.

Similar salt mixtures, suitable for direct addition to fresh water, are available from several commercial suppliers, but the cost of these mixtures is almost the same as the highest costs shown above.

[2] All volumes of water are expressed in imperial gallons.

Table 2: Composition and cost of artificial salt mixture

Common names of salts	Chemical composition	Range of costs at 1966 prices (per cwt)	Weight of each salt needed to make up the following weights of salt mixture									
			50 lb		100 lb		250 lb		500 lb	1 000 lb
			lb	oz	lb	oz	lb	oz	1b	lb
Sodium chloride (common salt)	NaCl	12s. 0d.–15s. 0d.	32	14	66	0	165	0	330	660
Magnesium sulphate (Epsom salt)	$MgSO_4$ $7H_20$	26s. 6d.–39s. 9d.	8	2	1	4	41	0	82	164
Magnesium chloride	$MgCl_2$ $6H_20$	25s. 6d.–46s. 0d.	6	8	13	0	33	0	66	132
Flake calcium chloride	$CaCl_2$ $2H_20$	34s. 6d.–80s. 6d.	1	12	3	8	9	0	18	36
Potassium chloride	KCl	46s. 6d.–87s. 6d.		14	1	12	4	8	9	18

Notes:

(a) Always specify both the name and the chemical composition when ordering, for there are several compounds having the same name but different chemical composition.

(b) Common salt should be of "pure vacuum dried" or cooking quality. Rock salt is not satisfactory.

(c) If flake calcium chloride is not available, hydrated calcium chloride ($Ca\ Cl_2\ 6H_20$) may be used, but the weight should be increased by 50 per cent, i.e. for 50 lb of salt mixture 2 lb 10 oz are required. Do not use anhydrous calcium chloride.

Table 3a, b and c: Composition of artificial seawater for use in lobster storage and shellfish purification units (for further details see Table 2)

Common names of salts	Weight of salts required by the following volumes of water											
	50 gal		100 gal		250 gal		500 gal		1 000 gal	1 litre
	lb	oz	lb	oz	lb	oz	lb	oz	lb	g
	(a) For lobster storage									
Sodium chloride	11	11½	23	8	58	8	117	0	235	23.51
Magnesium sulphate	2	14	5	12	14	8	28	8	57	5.77
Magnesium chloride	2	4½	4	9	11	8	23	0	46	4.58
Flake calcium chloride		9½	1	3	3	0	6	0	12	1.20
Potassium chloride		4½		9	1	4	3	0	6	0.57
TOTAL	17	12	35	9	88	12	117	8	356	35.63
These mixtures will give artificial seawater having a salinity of approximately 30‰										
	(b) For purification of native oysters									
Sodium chloride	10	9	21	1½	52	8	105	8	211	21.17
Magnesium sulphate	2	9½	5	3	13	0	26	0	52	5.20
Magnesium chloride	2	1	4	1½	10	4	20	8	41	4.12
Flake calcium chloride		8½	1	1	2	12	5	8	11	1.08
Potassium chloride		4		8	1	4	2	8	5	0.52
TOTAL	16	0	31	15	79	12	160	0	320	32.09
These mixtures will give artificial seawater having a salinity of approximately 27‰										
	(c) For purification of Portuguese oysters and hard clams									
Sodium chloride	8	9½	17	3½	43	0	86	0	172	17.25
Magnesium sulphate	2	1½	4	3½	10	8	21	0	42	4.24
Magnesium chloride	1	11	3	5½	8	4	16	8	33	3.36
Flake calcium chloride		7		14	2	4	4	8	9	0.88
Potassium chloride		3½		6½	1	0	2	0	4	0.42
TOTAL	13	0½	26	1	65	0	130	0	260	26.15
These mixtures will give artificial seawater having a salinity of approximately 22‰										

5. HOW TO MAKE UP ARTIFICIAL SEAWATER

The volume of the tank should be checked by making measurements of the length, breadth and average depth of the water, taking into account any irregularities of the

internal shape and also water in channels, pipes, etc. The volume in gallons may be obtained by multiplying the total volume in cubic feet by 6¼. Where small prefabricated tanks are used it is important to check their volume, for the nominal capacity, i.e. that given by the manufacturer, is often very different from the actual working capacity. It is also inadvisable to estimate the volume of an installation from the time taken to fill it with a pump whose flow is not accurately known; the actual pumping rate seldom coincides with that given by the manufacturer, on account of the method of installation and a general reduction in the efficiency of pumps with age. Having determined the water volume, the weight of salts required in the tank is gallons of water for use in lobster storage tanks, the weight of salts may be obtained by adding together the weights shown under the columns for 500, 250 and 50 gallons in Table 3(a).

The salts may be weighed out in a quantity suitable for one filling, or for several fillings, but, in the latter case, care must be taken to ensure that the minor salts are evenly distributed throughout the mixture. This difficulty can be overcome by keeping down the bulk and mixing together all the salts except the common salt, which is then added to the tank in the appropriate amount at the same time as the mixture. Salt mixture not used immediately should be stored in clean, dry containers. Before, during or after filling the tanks with water, the salts should be distributed throughout the tanks in a thin layer, beneath the inlet or near the outlet(s) of the circulating system, in order to speed up solution. Most of the salts will pass into solution rapidly but a small quantity may remain to form a fine white precipitate which may take several hours to disappear. When the bulk of the salts have dissolved, the salinity should be check with a hydrometer, and if satisfactory the shellfish may be immersed.

Water used for making artificial seawater should be of drinking quality. If any excessive quantity of chlorine is present, this will escape to the atmosphere during circulation. Extremely acid water, such as that from a peat catchment area or from certain mountainous areas, may be unsuitable for oyster purification, and, in cases of doubt, advice should be sought from the chemist of the local water undertaking. Artificial seawater for oyster purification should have a pH not less than 6.5.

6. THE USE OF SALTS FOR INCREASING THE SALINITY OF NATURAL SEAWATER

In estuaries and inlets which receive substantial quantities of fresh water, the salinity may at times fall below the minimum required for shellfish. Where a new installation is planned, the tank should be sited so that water of high salinity can be obtained at all times of the year, and for this purpose the proposed site should be examined during a wet spell, for water at a point which is of "full" salinity in summer may fall to 20‰ or lower during a prolonged wet spell. Whenever possible, salinity measurements should be made on samples taken at neap and spring tides from the same position and depth as the proposed intake; visual examination of the site without reference to salinity measurements may later lead to disappointment, for there is a tendency to underestimate the effect of fresh water in the lower parts on an estuary.

At the established installations, water of the highest salinity can usually be obtained during the last hour of the flood tide, and it is usually of a considerably higher salinity during the period of spring tides than on neaps. In places where the catchment area is a long way from the estuary the effect of heavy rain may not show in an estuary until several days later; after a period of heavy rain there is usually further delay before the salinity returns to normal. Where there are persistently low salinities, consideration should be given to extending the water intake to low-water mark, or even to a deep-water channel if this is not too far away.

Table 4: Approximate weights of salt mixture required to increase the salinity of natural seawater in shellfish tanks

Observed salinity (‰)	Observed specific gravity at temperature of			Weight of salt mixture for 100 gal, Made up according to Table 2					
	Up to 50°F (10°C)	51-59°F (10.1-15°C)	60°F (15.1°C) and above	Lobsters		Native oysters		Portuguese oysters and hard clams	
				lb	oz	lb	oz	lb	oz
27	1.023	1.022	1.021	-	-	-	-	-	-
26	1.022	1.021	-	1	3	-	-	-	-
25	1.021	-	1.020	2	6	1	3	-	-
24	1.020	1.020	1.019	3	9	2	6	-	-
23	-	1.019	1.018	4	12	3	9	-	-
22	1.019	1.018	-	5	15	4	12	-	-
21	1.018	1.017	1.017	7	2	5	15	-	-
20	1.017	-	1.016	8	5	7	2	1	3
19	1.016	1.016	1.015	9	8	8	5	2	6
18	-	1.015	1.014	10	11	9	8	3	9
17	1.015	1.014	-	11	14	10	11	4	12
16	1.014		1.013	13	1	11	14	5	15
15	1.013	1.013	1.012	14	4	13	1	7	2
14	1.012	1.012	1.011	15	7	14	4	8	5
13	-	1.011	-	16	10	15	7	9	8
12	1.011	-	1.010	17	13	16	10	10	11
11	1.010	1.010	1.009	19	0	17	13	11	14
10	1.009	1.009	1.008	20	3	19	0	13	1
9	1.008	1.008	-	21	6	20	3	14	4
8	-	1.007	1.007	22	9	21	6	15	7
7	1.007	-	1.006	23	12	22	9	16	10
6	1.006	1.006	1.005	24	15	23	12	17	13
5	1.005	1.005	1.004	26	2	24	15	19	0
4	-	1.004	-	27	5	26	2	20	3
3	1.004	-	1.003	28	8	27	5	21	6
2	1.003	1.003	1.002	29	11	28	8	22	9
1	1.002	1.002	1.001	30	14	29	11	23	12
0	-	-	-	32	1	30	14	24	15

When water temperature is not known, use the column showing SG at the lowest temperature range.

When existing pipe lines are extended, the rate of pumping may be substantially reduced by the friction of the longer pipe unless the pipe is of adequate diameter. The intake should be located on or near the seabed so as to take advantage of water of the highest salinity, and as far from sewage and industrial outfalls as possible. Outfalls containing gas-works liquors can be particularly troublesome, because extremely small quantities of these effluents in water taken into shellfish tanks can lead to the development of tasted similar to those of some disinfectants.

When water of low salinity is taken into an installation, the natural salt content may be increased by the addition of the salt mixture shown in Table 2. As a quick guide to the weight of salt mixture needed for raising the salinity, the following table show the weights of salts that must be added for every unit of salinity (1‰) or SG (0.001) that the water is below the recommended value.

To increase salt content by 1 unit of	Weight of salt mixture to be added to				
	100 gallons		1 000 gallons		1 cubic metre
	lb	oz	lb	oz	kg
Salinity (‰)	1	3	12	0	1.19
Specific gravity (0.001)	1	7	14	8	1.42

To increase the salinity of water from 15‰ to 20‰, (20-15 = 5) x 1 lb 3 oz = 6 lb of salt mixture must be added to every 100 gallons of water. If only the specific gravity is known, then to increase water from 1.016 to 1.020, each 100 gallons will require 1.020-1.016 = 4 units of SG) x 1 lb 7 oz = 5¾lb of salt.

Further details of the quantities of salt mixture required to make up the salinity under various conditions are given in Table 4. When the salinity of the water in an installation is known, the approximate weights of salts needed in tanks holding lobsters and oysters are shown on the same horizontal line on which the observed salinity appears, i.e. a lobster tank holding water of salinity 15‰ requires 14 lb 4 oz of salt mixture for every 100 gallons held in the tank. Alternatively, if the specific gravity and temperature are known, first the observed SG should be found under the appropriate temperature column, and then the weight of salts required for 100 gallons is given on the same horizontal line. For example, for Native oysters, water of SG 1.018 at 45 °F required 5 lb 5 oz for each 100 gallons to make it up to the desired SG of 1.022. If the water temperature is not known, then the observed specific gravity should be found in the second column headed "Up to 50 °F" and the weight of salts read off against this value, under the appropriate heading.

When water in lobster storage units is just below the required salinity it is possible to increase the salinity by the addition of common salt (sodium chloride) only. It is essential that the salt balance is not altered too much, and it is recommended that the use of common salt by itself be restricted to waters having an SG of 1.019 or more; for waters of lower salinity, the full salt mixture should be added. The salinity of water for use in oyster purifiction plants should be increased by the addition of the full salt mixture shown in Table 2, for it is essential that the oysters not only remain alive, but continue to function actively, so that purification can take place.

7. THE PLANNING OF NEW INSTALLATIONS OR THE EXTENSION OF EXISTING ONES

In installations which hold shellfish, the availability of water of adequate salinity at all times is of prime importance. Care taken in the selection of a site can save considerable cost later, particularly where tanks holding large volumes of water are involved. For this purpose, salinity surveys can be speeded up by the use of more advanced equipment than that described here.

For problems concerned with salinity, or with the design and construction of installations in which shellfish are stored or purified, the staff of the Ministry's Fisheries Laboratories at Conway (North Wales) and Burnham-on=Crouch (Essex) are available for consultation.

For those who need advice on how to store lobsters or purify oysters or mussels the following publication may be of assistance:

"Lobster storage" by H.J. Thomas. Available from HMSO, Edinburgh, price 1s. 6d.

"Handling lobsters and crabs" by H.J. Thomas. Available from Department of Agriculture and Fisheries for Scotland, Marine Laboratory, Aberdeen

"Refrigerated storage of lobsters" by H.J. Thomas. Scottish Fisheries Bulletin, No. 17, pp. 16–20. Available from HMSO Edinburgh.

"Lobster storage and shipment" by D.W. McLeese and D.G. Wilder. Available from the Queen's Printer, Ottawa, Canada price $1.75.
(This publication deals with lobster storage in Canada).

"The principles of water sterilization by ultra-violet light and their application in the purification of oysters" by P.C. Wood. Available from HMSO, London, price GBP 1.

"The purification of oysters in installations using ultra-violet light", Laboratory Leaflet No. 27. Available from the Fisheries Laboratory, Burnham-on-Crouch, Essex.

"A simplified system of mussel purification" by N. Reynolds. Available from HMSO, London, price 5s. 0d.

SUMMARY OF THE IMPORTANT POINTS

1. Minimum salt content of seawater

Shellfish	Minimum salinity ‰	Minimum SG (Rough guide)
Lobster	27.0	1.023
Native oysters	25.5	1.022
– Portuguese oysters	20.5	1.018
– Hard clams	20.0	1.017
Mussels	19.0	1.016

2. Artificial seawater

To make up artificial seawater (composition as in Table 2)

Shellfish	Weight of salt mixture for		Details
	100 gal	1 000 gal	
	lb oz	lb	
Lobsters	35 9	356	Table 3 (a)
Native oysters	31 15	320	Table 3 (b)
Portuguese oysters & hard clams	26 1	260	Table 3 (c)

To increase salinity of natural seawater

	Weight of salt mixture for 100 gal		Details
	lb	oz	
For each unit of salinity ‰ that is required	1	3	Page 12[3]
For each unit of SG (0.001) That is required	1	7	Page 12[3]

3. Use of common salt instead of complete salt mixture

Add to water in lobster storage tanks when SG is 1.019 or more. Do not use in shellfish purification tanks.

[3] See pages 122–123 in this document

Appendix 7

ENUMERATION OF *ESCHERICHIA COLI* IN MOLLUSCAN BIVALVE SHELLFISH

The Centre for Environment, Fisheries & Aquaculture Science (Cefas) – United Kingdom

European Community Reference laboratory for monitoring bacteriological and viral contamination of bivalve molluscs

GENERIC STANDARD OPERATING PROCEDURE

Issued by Technical Manager, Microbiological Food Safety

Authors' note: This generic standard operating procedure is based on ISO TS 16649-3. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide.

The Technical Specification is given in EU Regulations as the reference method for the enumeration of *E. coli* in live bivalve molluscs and should be used directly by laboratories that need to ensure that they comply fully with the method for the purposes of testing in accordance with legislation. The Generic Standard Operating Procedure is given for information only.

INDEX

HISTORY OF PROCEDURE

Controlled document title: ENUMERATION OF *ESCHERICHIA COLI* IN MOLLUSCAN BIVALVE SHELLFISH

Controlled document reference: SOP 1175

Issue number	Date issued	Sections involved
1	22.03.01	All
2	03.04.01	All
3	02.05.01	All
4	15.05.03	All
5	05.02.07	All
6	16.11.07	All
7	04.04.08	Table 2

1.0 INTRODUCTION

Infectious human diseases acquired from the consumption of bivalve molluscan shellfish are internationally recognised. These health hazards are largely due to the phenomenon of filter-feeding where-by bivalve molluscs concentrate and retain bacterial and viral pathogens often derived from sewage contamination of their growing waters. The risks of exposure to infectious agents are compounded by the traditional consumption of bivalve shellfish raw, or only lightly cooked. Historically, enteric bacteria, such as faecal coliforms, have been adopted as surrogate indicator organisms to assess the quality of shellfish flesh, and, consequently, to predict the risk of exposure to enteric pathogenic viruses.

In the European Union, the criteria for laying down the microbiological standards for bivalve molluscs are set out in Regulation (EC) 854/2004 (Anon 2004) and Regulation (EC) 2073/2005 (Anon 2005) stipulating conditions for the production and placing on the market of live bivalve molluscan shellfish. In the United Kingdom *Escherichia coli* is used as an indicator of faecal contamination of bivalve molluscan shellfish.

2.0 SCOPE

The procedure has been produced with reference to ISO TS 16649-3 (Anon 2005). The theoretical limit of detection is a most probable number (MPN) of 20 *E. coli* per 100g of shellfish flesh. In the context of this test *E. coli* produces acid from lactose at 37±1°C and expresses β-glucuronidase activity at 44±1°C.

Note: The 5x3 MPN tables included in this procedure are taken from ISO 7218:2007 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations'.

3.0 PRINCIPLE

The method used to enumerate *E. coli* in molluscan shellfish is a two-stage, five-tube three-dilution most probable number (MPN) method. The first stage of the method is a resuscitation step requiring inoculation of minerals modified glutamate broth (MMGB) with a series of diluted shellfish homogenates and incubation at 37±1°C for 24±2 hours. The presence of *E. coli* is subsequently confirmed by subculturing acid producing tubes onto agar containing 5-bromo-4-chloro-3-indolyl-β-D glucuronide and detecting β-glucuronidase activity.

4.0 SAFETY PRECAUTIONS

Standard microbiology safety precautions should be applied throughout. Risks of cuts and minor physical injury exist when performing this procedure, particularly when using sharp oyster knifes to open shellfish. Appropriate measures to reduce these risks should be taken. Homogenisation of shellfish should be performed in a laminar flow cabinet to reduce the risk of infection from aerosol inhalation. *E. coli* should be handled in accordance with ACDP category 2 guidelines.

5.0 EQUIPMENT

- Waring blender and jars
- Stomacher
- Stomacher bags
- Laminar air flow cabinet (Class ll)
- Refrigerator at 3±2˚C
- Sterile glassware
- Shucking knife
- Safety/electric Bunsen system
- Latex gloves
- Safety gloves
- Incubator at 37±1˚C
- Incubator at 44±1˚C
- Loops - sterile, 1µl and10µl
- Pipette - automatic or manual for use with 1ml and 10ml open ended pipette tips

6.0 MEDIA AND REAGENTS

- Ethanol
- 0.1% peptone water; formula per litre - de-ionised water 1±0.01 litre, peptone bacteriological (Oxoid LP37) 1.0±0.1g
- Minerals modified glutamate broth (MMGBx1, MMGBx2); - Single strength - de-ionised water 1±0.01 litre, ammonium chloride (Merck) 2.5±0.1g, sodium glutamate (Oxoid L124) 6.4±0.1g, minerals modified medium base (Oxoid CM607) 11.4±0.1g. Double strength - de-ionised water 1±0.01 litre, ammonium chloride (Merck) 5.0±0.1g, sodium glutamate (Oxoid L124) 12.8±0.1g, minerals modified medium base (Oxoid CM607) 22.8±0.1g, pH 6.7±0.1
- Tryptone bile glucuronide agar (TBGA); formula per litre - de-ionised water 1±0.01 litre, tryptone bile glucuronide agar (Lab M) 36.5±0.5g, pH 7.2±0.2

7.0 MICROBIOLOGICAL REFERENCE MATERIALS

7.1 Mineral-modified glutamate medium (MMGB) performance testing
Escherichia coli ATCC 25922 or ATCC 8739 - acid production
Enterococcus faecalis ATCC 29212 or ATCC 19433 - no growth

7.2 Tryptone bile glucuronide agar (TBGA) performance testing
Eschericia coli ATCC25922 or 8739 – β-glucuronidase positive
Escherichia coli NCTC 13216 – β-glucuronidase positive (weak)
Enterococcus faecalis ATCC 29212 or ATCC 19433 - no growth

8.0 PROCEDURE

8.1 Sample receipt

Samples must be received in an intact food grade plastic bag and properly packed in a cool box with ice packs – packed in this manner they should reach a temperature of less than 8°C within 4 hours and then maintain this for at least 24 hours. Such samples should not be received frozen. Samples from harvesting areas should have been rinsed, but not immersed, and drained at time of sampling and should be regarded as unsatisfactory when they are received in the laboratory if the sample container is leaking, the shellfish are covered in mud or immersed in water or mud/sand.

8.2 Sample storage

Upon receipt in the laboratory the temperature of the samples should be recorded. Samples should preferably be examined immediately - if storage in the laboratory is necessary then this should be done at 3±2°C and no more than 24 hours should elapse between sample collection and commencement of the test. However, this may be extended to 48 hours where maintenance of the required temperature has been formally validated for the full 48 hour period under normal sampling and sample transport conditions. Samples for *E. coli* analysis should not be frozen.

8.3 Sample selection

Choose shellfish that are alive according to the following points:

- If any flesh is exposed and reacts to touch using a sterile shucking knife with movement of any kind.
- If the shellfish are open and then close of their own accord.
- If a tap on the shell causes closing or movement.
- Tightly closed shellfish.

Discard all dead shellfish and those with obvious signs of damage. Select the appropriate number of shellfish depending on the species (Appendix 1). More shellfish can be used, if necessary, to produce the required volumes for each analysis.

8.4 Sample preparation

Mud and sediment adhering to the shellfish should be removed prior to opening the shellfish by rinsing/scrubbing under cold, running tap water of potable quality. Shellfish should not be re-immersed in water as this may cause them to open. Open all selected shellfish as described below with a flame sterilised shucking knife and empty meat and liquor into a beaker. To flame sterilise the shucking knife place the knife in the beaker of ethanol and sterilise using an electric Bunsen system. Allow the knife to cool before using. When opening shellfish ensure that the hand holding the shellfish is protected with a heavy-duty safety glove to prevent cuts.

8.4.1. Oysters and clams

Insert the knife between the two shells towards the hinge end of the animal. Push the knife further into the animal and prise open the upper shell, allowing any liquor to drain into the beaker. Push the blade through the animal and sever the muscle attachments by sliding across the animal. Remove the upper shell and scrape the contents of the lower shell into a beaker.

8.4.2. Mussels and cockles

Insert the knife in between the shells of the animal and separate the shells with a twisting motion of the knife. Collect the liquor from the animal in the beaker then cut the muscle between the shells and scrape the contents into a beaker.

8.5 Dilution and homogenisation

Weigh the beaker and calculate the weight of the contents by subtracting the weight of the pre-weighed beaker to the nearest gram. Add 2ml of sterile 0.1% PW per 1g of shellfish using a measuring cylinder and measure to ±2ml.

Note: Complete either sections 8.5.1 or 8.5.2.

8.5.1. Blending

Place contents of beaker into a 1 litre blender jar[1] and homogenise at high speed for approximately 1 minute (4 bursts of 15 seconds with at least 5 seconds between bursts) in a class two microbiological laminar flow cabinet. Decant the contents back into the labelled beaker.

8.5.2. Stomaching

If a stomacher (peristaltic homogeniser) is used, the initial homogenisation should be done using a proportion of the volume of diluent calculated, and the resultant homogenate added to the rest of the calculated volume and thoroughly mixed. Place the contents of the beaker into at least three stomacher bags, to avoid small pieces of shell from puncturing the bags. Remove excess air from the bag. Operate the stomacher for 2-3 minutes.

Add 30±0.5ml of mixed shellfish homogenate to 70±1ml of 0.1% PW using a 10ml open-ended pipette to make a master 10^{-1} dilution. Thoroughly mix by vigorous shaking of the bottle. Make further dilutions to 10^{-2} in 0.1% PW or if samples are expected to be heavily polluted (Category C or above) further decimal dilutions as necessary.

8.6 Inoculation and incubation of primary broth

Inoculate five bottles containing double strength MMGB with 10±0.2ml of the 10^{-1} diluted homogenate (equivalent to 1g of tissue per tube). Inoculate five bottles single strength MMGB with 1±0.1ml of the 10^{-1} diluted homogenate. Inoculate five bottles single strength MMGB with 1±0.1ml of the 10^{-2} diluted homogenate and repeat with any further dilutions. Inoculate an individual universal bottle of single strength MMGB for *E. coli* ATCC 25922 or ATCC 8739 and *E. faecalis* ATCC 29212 or 19433 using a 10µl loop. Inoculate one bottle of single strength MMGB uninoculated. Incubate inoculated bottles of MMGB at 37±1˚C for 24±2 hours.

8.7 Confirmation of *E. coli*

After incubation examine the MMGB for the presence of acid. Acid production is denoted by the presence of any yellow coloration throughout the medium. Confirm the presence of *E. coli* in tubes showing acid production by subculture onto tryptone bile glucuronide agar (TBGA) media within 4 hours, streaking to obtain single colonies. Inoculate one TBGA plate with *E. coli* ATCC 25922 or ATCC 8739, *E. coli* NCTC 13216 and *E. faecalis* ATCC 29212 or ATCC 19433. Incubate TBGA at 44±1˚C for 22±2 hours.

After the incubation period examine the TBGA for the presence of blue-green colonies. Record the results as '+' (positive) for any shade of dark or light blue or blue-green colonies, '-' (negative) for colonies of any other colour and 'NG' for no growth.

8.8 Calculation of *E. coli* most probable number and reporting

To calculate the most probable number (MPN), record the number of TBGA plate positives for each dilution. This gives a three figure tube combination number, which

[1] If shellfish are particularly small it may be necessary to use a smaller blender to achieve a consistent homogenate.

is used to calculate the MPN. MPN tube combinations fall into one of four categories. 95% of observed tube combinations fall in to category 1 with 4%, 0.9% and 0.1% in categories 2, 3 and 0 respectively. Both the category and MPN result can be determined from the MPN table (see Appendix 2) as follows:

From the three figure number derived from the combination of positive results look up the MPN result using the MPN tables, (see Appendix 2), as follows:

- For dilutions of neat, 10^{-1} and 10^{-2} use MPN Table 1.
- For dilutions of 10^{-1}, 10^{-2} and 10^{-3} use MPN Table 2.
- For dilutions of 10^{-2}, 10^{-3} and 10^{-4} use MPN Table 3.
- For greater dilutions use MPN Table 3 and multiply the result by the extra number of dilution factors.

Where more than three dilutions have been tested for a sample, select the tube combination as stated in the following rules:

1. Select the combination of three consecutive dilutions having a category 1 profile to obtain the MPN index. If more than one combination having a category 1 profile is obtained, use the one with the highest number of positive tubes.

2. If no combination having a category 1 profile is available, use the one having a category 2 profile. If more than one combination having a category 2 profile is obtained, use the one with the highest number of positive tubes.

Adapted from: ISO 7218:2007

Results should be reported as the most probable number per 100g of shellfish. Negative samples should be reported as MPN <20/100g. Where the MPN tube combination is not given in the relevant table, the result should be reported as 'Void'.

Note: The 5-tube 3-dilution MPN table given in ISO 7218:2007 includes all category 1 and category 2 combinations, and some (but not all) category 3 combinations. A note is included in the standard that: "Before starting testing, it should be decided which category will be acceptable, that is, only 1, 1 and 2 or even 1, 2 and 3. When the decision to be taken on the basis of the result is of great importance, only category 1, or at most 1 and 2, results should be accepted. Category 0 results should be considered with great suspicion". Given that the NRL generic SOP will be referred to by official control laboratories, all of the category 3 combinations have been omitted from the version of the tables presented here.

9.0 UNCERTAINTY OF TEST RESULTS

Uncertainty inherent in any test method, i.e. instruments, media, analyst performance etc can be assessed by the repeatability and reproducibility of test results. These should be monitored through control tests analysed alongside sample tests, through in-house comparability testing between analysts and through external intercomparison exercises, which would highlight any uncertainties within the test methods.

10.0 REFERENCES

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11.0 APPENDICES

11.1 Appendix 1: sub-sample sizes of shellfish required for *E. coli* analysis

The following sub-sample sizes are recommended for inclusion in the homogenisation step:

King scallops (*Pecten maximus*)	10–12
Horse mussels (*Modiolus modiolus*)	10–12
Sand Gapers (*Mya arenaria*)	10–12
Razor clams (*Ensis* spp.)	10–12
Oysters (C*rassostrea gigas* and O*strea edulis*)	12–18
Hard clams (*Mercenaria mercenaria*)	12–18
Queen scallops (*Aequipecten opercularis*)	15–30
Mussels (*Mytilus* spp.)	15–30
Manila clams (*Tapes philippinarum*)	18–35
Palourdes (*Tapes decussatus*)	18–35
Cockles (*Cardium edule*)	30–50
Thick trough shells (*Spisula solida*)	30–50

The weight of shellfish flesh and liquor must be at least 50g for the *E. coli* method. For species not given in the table, sufficient shellfish should be opened to achieve this minimum weight of flesh and liquor, with the proviso that a minimum of ten animals should be used for very large species such as *Mya*. In general, the more shellfish that are included in the initial homogenate, the less the final result will be influenced by the inherent animal-to-animal variation in *E. coli* concentration.

11.2 Appendix 2: *E. coli* most probable number (MPN) tables

11.2.1 Table 1: Most probable number of organisms: table for multiple tube methods using 5 × 1 g, 5 × 0.1 g, 5 × 0.01 g.

1g	0.1g	0.01g	MPN/100g	Category
0	0	0	<20	–
0	1	0	20	2
1	0	0	20	1
1	0	1	40	2
1	1	0	40	1
2	0	0	50	1
2	0	1	70	2
2	1	0	70	1
2	1	1	90	2
2	2	0	90	1
3	0	0	80	1
3	0	1	110	1
3	1	0	110	1
3	1	1	140	2
3	2	0	140	1
3	2	1	170	2
3	3	0	170	2
4	0	0	130	1
4	0	1	170	1
4	1	0	170	1
4	1	1	210	1
4	2	0	220	1
5	0	0	230	1
4	2	1	260	2
4	3	0	270	1
4	3	1	330	2
4	4	0	340	2
5	0	1	310	1
5	1	0	330	1
5	1	1	460	1
5	1	2	630	2
5	2	0	490	1
5	2	1	700	1
5	2	2	940	2
5	3	0	790	1
5	3	1	1 100	1
5	3	2	1 400	1
5	4	0	1 300	1
5	4	1	1 700	1
5	4	2	2 200	1
5	4	3	2 800	2
5	4	4	3 500	2
5	5	0	2 400	1
5	5	1	3 500	1
5	5	2	5 400	1
5	5	3	9 200	1
5	5	4	16 000	1
5	5	5	>18 000	–

11.2 *E. coli* most probable number (MPN) tables

11.2.2 Table 2: Most probable number of organisms: table for multiple tube methods using 5 × 0.1 g, 5 × 0.01 g, 5 × 0.001 g.

0.1g	0.01g	0.001g	MPN/100g	Category
0	0	0	<200	–
0	1	0	200	2
1	0	0	200	1
1	0	1	400	2
1	1	0	400	1
2	0	0	500	1
2	0	1	700	2
2	1	0	700	1
2	1	1	900	2
2	2	0	900	1
3	0	0	800	1
3	0	1	1 100	1
3	1	0	1 100	1
3	1	1	1 400	2
3	2	0	1 400	1
3	2	1	1 700	2
3	3	0	1 700	2
4	0	0	1 300	1
4	0	1	1 700	1
4	1	0	1 700	1
4	1	1	2 100	1
4	2	0	2 200	1
5	0	0	2 300	1
4	2	1	2 600	2
4	3	0	2 700	1
4	3	1	3 300	2
4	4	0	3 400	2
5	0	1	3 100	1
5	1	0	3 300	1
5	1	1	4 600	1
5	1	2	6 300	2
5	2	0	4 900	1
5	2	1	7 000	1
5	2	2	9 400	2
5	3	0	7 900	1
5	3	1	11 000	1
5	3	2	14 000	1
5	4	0	13 000	1
5	4	1	17 000	1
5	4	2	22 000	1
5	4	3	28 000	2
5	4	4	35 000	2
5	5	0	24 000	1
5	5	1	35 000	1
5	5	2	54 000	1
5	5	3	92 000	1
5	5	4	160 000	1
5	5	5	>180 000	–

11.2 *E. coli* most probable number (MPN) tables

11.2.3 Table 3: Most probable number of organisms: table for multiple tube methods using 5 × 0.01 g, 5 × 0.001 g, 5 × 0.0001 g.

0.01g	0.001g	0.0001g	MPN/100g	Category
0	0	0	<2 000	–
0	1	0	2 000	2
1	0	0	2 000	1
1	0	1	4 000	2
1	1	0	4 000	1
2	0	0	5 000	1
2	0	1	7 000	2
2	1	0	7 000	1
2	1	1	9 000	2
2	2	0	9 000	1
3	0	0	8 000	1
3	0	1	11 000	1
3	1	0	11 000	1
3	1	1	14 000	2
3	2	0	14 000	1
3	2	1	17 000	2
3	3	0	17 000	2
4	0	0	13 000	1
4	0	1	17 000	1
4	1	0	17 000	1
4	1	1	21 000	1
4	2	0	22 000	1
5	0	0	23 000	1
4	2	1	26 000	2
4	3	0	27 000	1
4	3	1	33 000	2
4	4	0	34 000	2
5	0	1	31 000	1
5	1	0	33 000	1
5	1	1	46 000	1
5	1	2	63 000	2
5	2	0	49 000	1
5	2	1	70 000	1
5	2	2	94 000	2
5	3	0	79 000	1
5	3	1	110 000	1
5	3	2	140 000	1
5	4	0	130 000	1
5	4	1	170 000	1
5	4	2	220 000	1
5	4	3	280 000	2
5	4	4	350 000	2
5	5	0	240 000	1
5	5	1	350 000	1
5	5	2	540 000	1
5	5	3	920 000	1
5	5	4	1 600 000	1
5	5	5	>1 800 000	–

World bivalve production and consumption has increased significantly in recent years, from a combined total for wild catch and aquaculture of approximately 10.7 million tonnes in 1999 to 14 million tonnes in 2006. Furthermore, the development of freight by air and sea and preservation techniques have enabled consumers, in different parts of the world, to enjoy eating bivalves produced in distant waters. Such developments in distribution and trade have in turn led to emerging challenges for consumer protection, particularly in relation to the safety of bivalves from pathogenic micro-organisms. Several species of bivalves are often consumed live or raw (e.g. oysters), or lightly cooked (e.g. mussels) which make them a high risk food product category requiring proper control measures to eliminate or reduce to acceptable levels potential biological, chemical and physical hazards. This document is intended to provide a basic introduction to the public health problems that can be associated with shellfish consumption and to provide guidance to the bivalve industry as to how a depuration centre, and the associated systems, should be planned, constructed and operated. It is mainly targeted at new operators or those with limited experience, as well as fishery and public health officers who deal with the bivalve industry. This is of particular importance for several developing countries, where the bivalve industry is expanding quickly with the aim of winning an ever larger share of the bivalve international market.

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