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

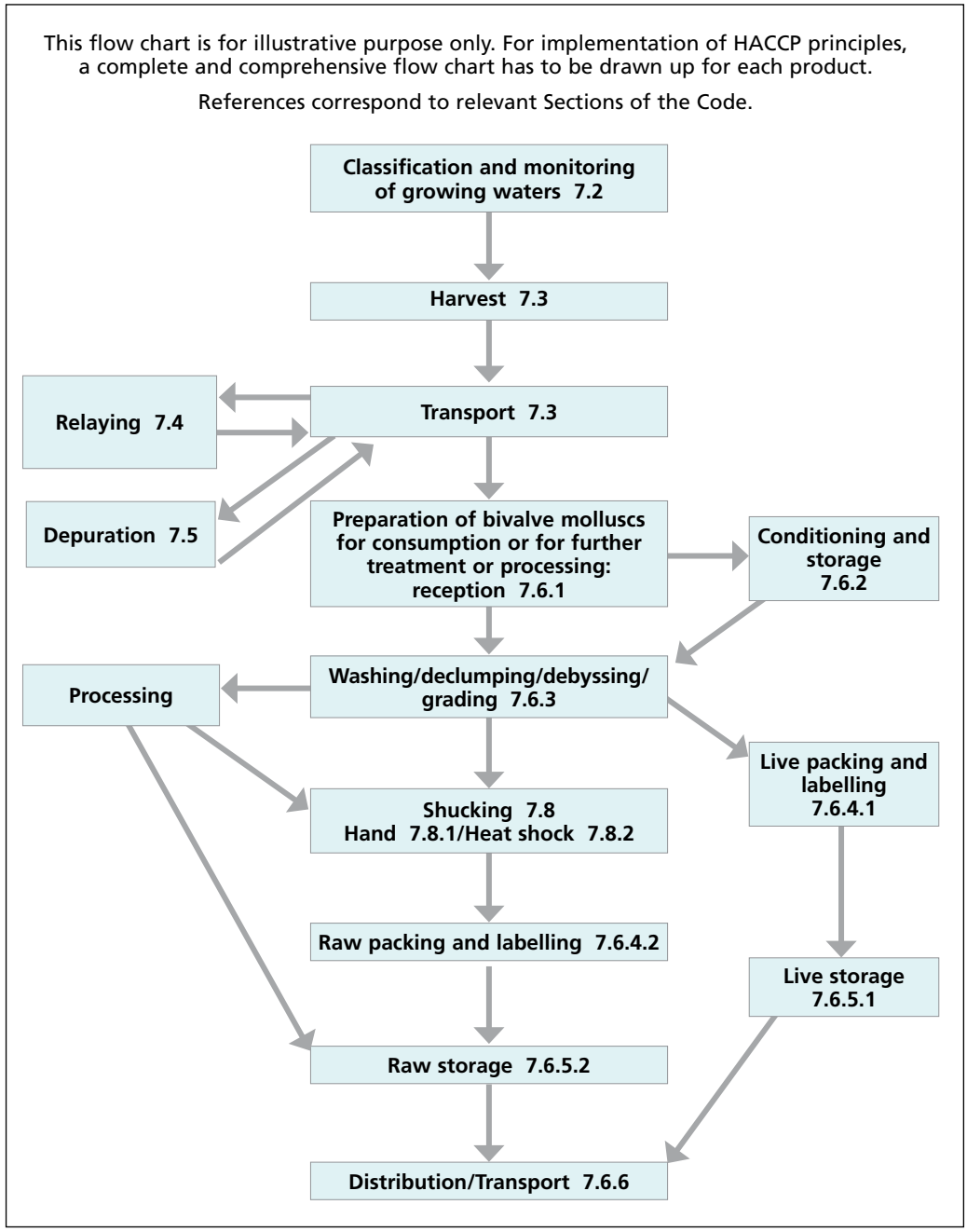
PROPOSED DRAFT CODE OF PRACTICE FOR FISH AND FISHERY PRODUCTS
(At Step 8 of the procedure)
ALINORM 07/30/18
APPENDIX IV

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.

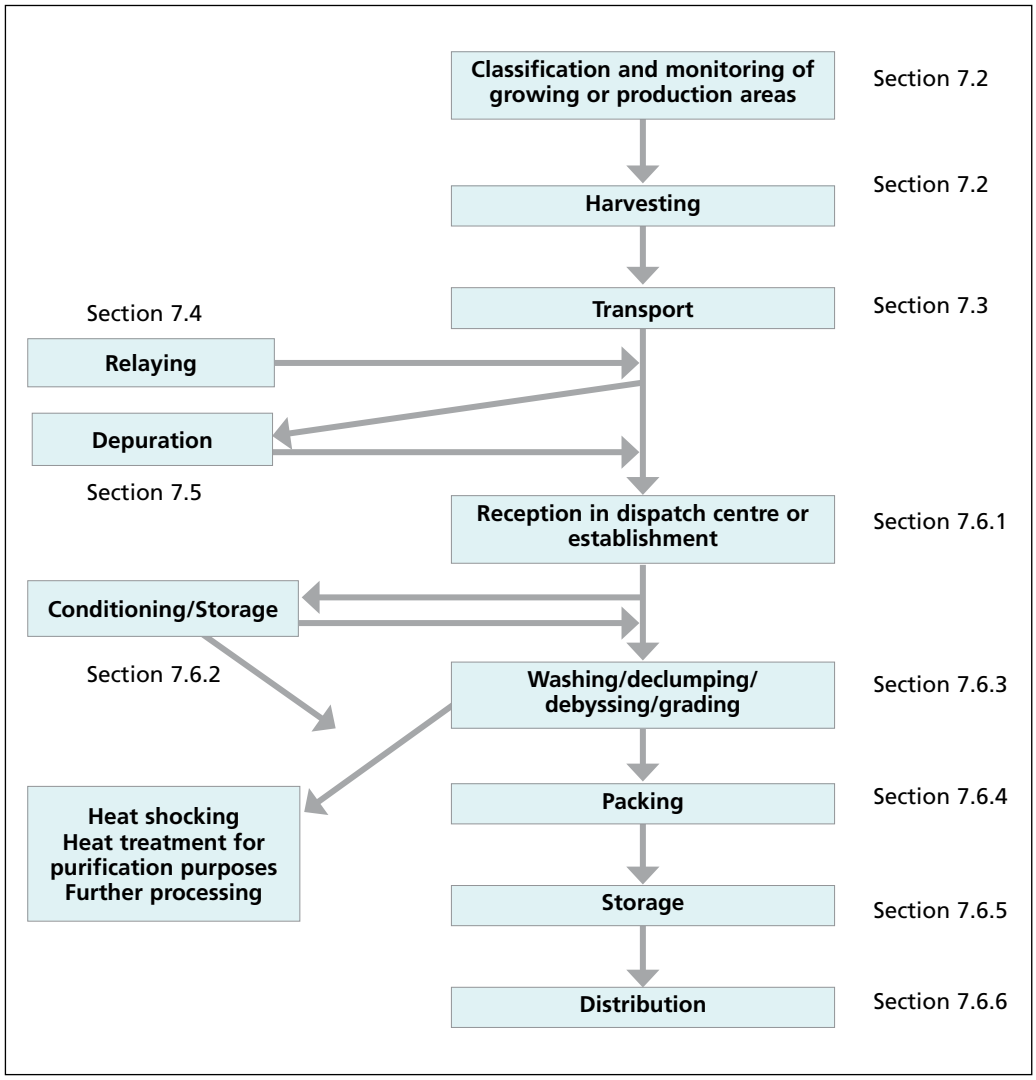


Figure 7.1: Example of a simplified flow diagram for the production of live and raw bivalve molluscs

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.

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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

Anon. 1999. ISO 6887-1:1999. 'Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions'.

Anon. 2004. Regulation (EC) No 854/2004 of the European parliament and the council, 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

Anon. 2004. ISO/TS 16649-3:2004. 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide'.

Anon. 2005. Commission Regulation (EC) No 2073/2005 of the European parliament and the council, 15 November 2005 on microbiological criteria for foodstuffs.

Anon. 2007. ISO 7218:2007, 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.

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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