TECHNICAL GUIDANCE
FOR THE DEVELOPMENT OF THE GROWING AREA ASPECTS OF BIVALVE MOLLUSC SANITATION PROGRAMMES

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CONTENTS

FIGURES

TABLES

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ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
BOD5	five-day biological oxygen demand
CAC	Codex Alimentarius Commission
CFU	Coliform-forming Unit
COP	[Codex] Code of practice
CSO	Combined Sewer Overflows
CTD	Conductivity, Temperature, Depth
DEM data	Digital Elevation Model data
EC	European Commission
EU	European Union
EURL	The Reference Laboratories of the European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FC	faecal coliform
GIS	Geographical Information System
GPS	Global Positioning System
HAV	hepatitis A virus
IQF	Individually Quick Frozen
ISO	International Organization for Standardization
LIMS	Laboratory Information Management System
MBR	Membrane bioreactor
MLLW	Mean Low Low Water
MOU	Memoranda of Understanding
MPN	Most probable number
MSC	Male-specific Coliphage
NOAA	United States National Oceanic and Atmospheric Administration
NGO	non-governmental organization
NoV	norovirus
NSSP	National Shellfish Sanitation Program (USA)

OIE	World Organisation for Animal Health
PFU	plaque-forming unit
RBC	Rotating Biological Contactors
SCADA	Supervisory Control And Data Acquisition
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures
STW	Sewage treatment works
TSS	totals suspended solids
UNICEF	United Nations Children's Fund)
USA	United States of America
USNSSP, U.S. NSSP	United States National Shellfish Sanitation Programme
UV	ultraviolet
WHO	World Health Organization
WTO	World Trade Organization
WWTP	Wastewater Treatment Plant
WWTW	Wastewater treatment works

INTRODUCTION

1.1 BACKGROUND/CONTEXT

International trade has been the main driving factor for the rapid growth of the bivalve mollusc production industry during the last six decades, growing from nearly one million tonnes in 1950 to 16.1 million tonnes in 2015. According to FAO statistics, the value of bivalve mollusc trade reached US$ 4.6 billion in 2014 and slightly declined to US$ 4.4 billion in 2015. Though bivalves are traded in different forms such as fresh, chilled, frozen or canned, the value of trade in live, fresh and chilled bivalves stood at US$ 1.2 billion in 2015. However, since bivalves are filter feeding organisms that can concentrate micro-organisms and chemicals from the environment around them, there are very stringent sanitary requirements for live and raw bivalve molluscs in international trade. According to Article 3 of the Agreement on the application of Sanitary and Phytosanitary (SPS) Measures under the World Trade Organization (WTO), members are to base their sanitary or phytosanitary measures on international standards, guidelines or recommendations. The international standard setting body recognized under the SPS agreement for food safety is the Codex Alimentarius Commission (CAC).

The Codex Alimentarius Commission has developed a Standard for Live and Raw Bivalve Molluscs. The Codex Code of Practice (COP) for Fish and Fishery Products (CAC, 2012a) has a section on the Processing of Live and raw bivalve molluscs (Section 7), which is intended to provide guidance on the steps needed to be taken at all stages of food chain in order to produce a product that meets the Codex Standard. The Code of Practice provides information on the prerequisite programmes, sanitary surveys, classification and monitoring of growing areas to take care of microbiological, chemical contamination and biotoxins[1]. Additional guidance on specific issues such as management of pathogenic *Vibrio* spp. (CAC, 2010a) and viruses (CAC, 2012b), are provided in CAC Guideline documents.

However, the guidance given in the Codex COP is very broad and does not specify the precise manner in which the classification of the bivalve mollusc growing area is to be established and monitored. For example, it does not indicate the faecal indicator bacteria to be chosen for the sanitary survey and monitoring; whether growing area

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[1] Poisonous substances naturally present in fish and fishery products or accumulated by the animals feeding on toxin-producing algae or in water containing toxins produced by such organisms.

classification should be based on faecal indicator levels in growing area waters or in bivalve mollusc samples; frequency of sampling; and quantifiable limits for the chosen indicator bacteria. This has resulted in different countries adopting different approaches. The United States of America (USA) and the European Union (EU) are major markets for bivalves, and these two major markets have different approaches for managing bivalve mollusc safety. Countries wanting to export bivalves to these two major markets are required to comply with both systems. Other major markets include China and Japan, and these have their own specific requirements for imports. The need to comply with different systems has constrained many countries from accessing multiple markets. Further, countries that are wishing to establish a bivalve mollusc sanitation programme for protection of their own consumers are constrained by a lack of clarity on the best approach to follow.

The need for developing international guidance for implementation of bivalve mollusc sanitation programme within the framework of the Section 7 of the Codex COP for Fish and Fishery Products was identified by the representatives of 15 major bivalve producing and trading countries participating in the 2nd International Workshop on Molluscan Shellfish Sanitation: Application of Sanitary Surveys, held 24–28 September, 2012, in Newport, USA. The 33rd Session of the Codex Committee on Fish and Fishery Products and the FAO Committee on Fisheries Sub-Committee on International Trade supported the development of international guidance by FAO/WHO.

The guidance has been developed by a team of International experts representing different geographical regions and different bivalve mollusc production practices. The development of the guideline has further benefitted by consultation with a larger group of experts and stakeholders attending the International Conference on Molluscan Shellfish Safety, held in Puerto Varas, Chile and in Galway, Ireland in 2015 and 2017 respectively.

1.2 SCOPE

The guidance is mainly intended for primary production of molluscs for consumption as live or raw bivalves. In this context, they apply to Section 7.2 of the COP. In addition, they apply to assessment and monitoring of areas used for relaying (Section 7.4 of the Code of Practice). Areas used for conditioning and wet storage (Section 7.6.2) in the natural environment may also be subject to assessment and monitoring and the same principles will apply. Consideration has been primarily given to general requirements and microbiological hazards. For chemical hazards, toxin phytoplankton and biotoxins, reference has been provided to relevant Codex standards and Codex and other international guidance addressing these hazards, where available (Lawrence, *et al.*, 2011; Ryder, Karunasagar and Ababouch, 2014). Where the same principles may apply to all types of contamination, the recommendations given in this guidance have been extended to cover all that may be applicable. In general, this relates to the Growing Area Risk Profile (Section 2), Growing Area Assessment (Section 3), Growing Area Management (Section 6) and Growing Area Review (Section 7).

A complete bivalve sanitation programme includes several other elements in addition to those relating to the growing area. These other elements are covered in Section 7 of the Codex COP and the Codex Standard for Live and Raw Bivalve Molluscs. They include:

> Harvesting and transportation;

> Relay;

> Depuration;

> Processing;

> Lot identification;

> Recall procedures;

> Composition and quality;

> including specified limits for contaminants and hygiene indicators; and

> Labelling and storage.

In addition, other requirements not related to food safety may need to be put in place to satisfy international trade requirements and may be relevant for consideration for production for domestic trade. One significant aspect is the monitoring and control of diseases of bivalve molluscs. Information on this aspect is available from the World Organisation for Animal Health (OIE; http://www.oie.int/), the European Union Reference Laboratory for Molluscs Diseases (http://www.eurl-mollusc.eu/) and the United States National Oceanic and Atmospheric Administration (NOAA; http://www.noaa.gov/).

It is recognized that bivalves harvested from freshwater environments (e.g. watercourses and lakes) may be consumed in some countries. However, the proportion consumed from such locations is small compared to that consumed from brackish and marine waters. This guidance does not specifically address the food safety aspects of freshwater bivalve growing areas. While many of the principles within the guidance can be used for such growing areas, there are some important differences, and some additional considerations. The development of a programme for a freshwater growing area would require appropriate expertise to address these aspects.

1.3 APPROACH TO DEVELOPMENT OF THE GUIDANCE

The guidance was developed from a technical and scientific perspective and using a risk-based approach. It has been driven by the intent of existing programmes, rather than the details of these programmes and an attempt has been made to map this to existing programmes and Codex Codes of Practice (COPs). Implementing a bivalve mollusc sanitation programme requires collaboration and agreements between different partners including local authorities, regulatory agencies and laboratories. The guidance starts by providing a framework to develop a risk profile, which serves as the primary assessment of the sanitary status for the area and leads to a

primary decision step as to whether further steps are warranted or whether the area is unsuitable for harvest for human consumption. If the decision is to proceed, the risk profile provides the basis for the next steps of growing area assessment, growing area monitoring and classification. These steps provide a fuller basis for assessment of the sanitary status and the framework for risk management of the growing area. The bivalve mollusc sanitation programme is iterative and the guidance also considers the necessity for ongoing review of the programme.

Due to the potential for changes to be made to the regulatory requirements applied by different countries, it is not possible to provide detailed cross-reference to those requirements within this guidance. However, the approach given in this guidance provides a framework that should ensure that most of the requirements are met: users should check any specific requirements necessary for trade or national purposes and ensure that any differing, or additional, items are addressed within the sanitation programme.

1.4 USE OF THE GUIDANCE DOCUMENT

Authorities responsible for the development, implementation and application of a bivalve mollusc sanitation programme will need to consider at each stage whether any specific requirements need to be met with regard to international trade (e.g. application of those requirements of the receiving country(ies) or existing national regulations. Such requirements may apply to the whole programme or one or more parts. It should be possible to apply such regulatory requirements within the framework of these guidelines. If there is a conflict, the regulatory requirements should be used. In the context of international trade, any arbitration by the World Trade Organization (WTO) will take into account the content of the relevant Codex standard.

Figure 1.1 shows the growing area programme process described in this guidance. The process is analogous to the components of a Risk Analysis. The initial Risk Profile is intended to ensure that the responsible authority, as the risk manager, makes an initial assessment in order to decide whether a programme is warranted for the area under consideration and, if so, what hazards need to be taken into consideration. The Growing Area Assessment and Growing Area Monitoring steps constitute a Risk Assessment. The Classification and Growing Area Management steps are both part of the Risk Management process. The Review step contains both Risk Assessment and Risk Management elements as all aspects of the programme are considered. Risk Communication is primarily addressed in the Risk Profile and Growing Area Management: however, it is important that communication with relevant stakeholders is undertaken during the other parts of a programme for a specific area.

Table 1.1 shows the relationship between the major sections of this guidance document and the Codex COP, and EU and US regulatory requirements and guidance. This is provided for information only and it is the responsibility of the responsible authority to identify all specific requirements that need to be met by a growing area programme undertaken for the purposes of international trade.

FIGURE 1.1 **PROCESS FLOW CHART FOR A GROWING AREA PROGRAMME**


```
                    INTENT TO HARVEST

                    PROCEED?              ──  no  ──▶   NO HARVEST ALLOWED¹
                        │
                       yes
                        ▼
                GROWING AREA ASSESSMENT

                 PRIMARY MONITORING

                    CLASSIFICATION        ──  category IV  ──▶   NO HARVEST ALLOWED¹
                   │              │
         category I.II or III   category I.II or III
                   ▼              ▼
         ONGOING MONITORING    GROWING AREA MANAGEMENT

                      REVIEW
```
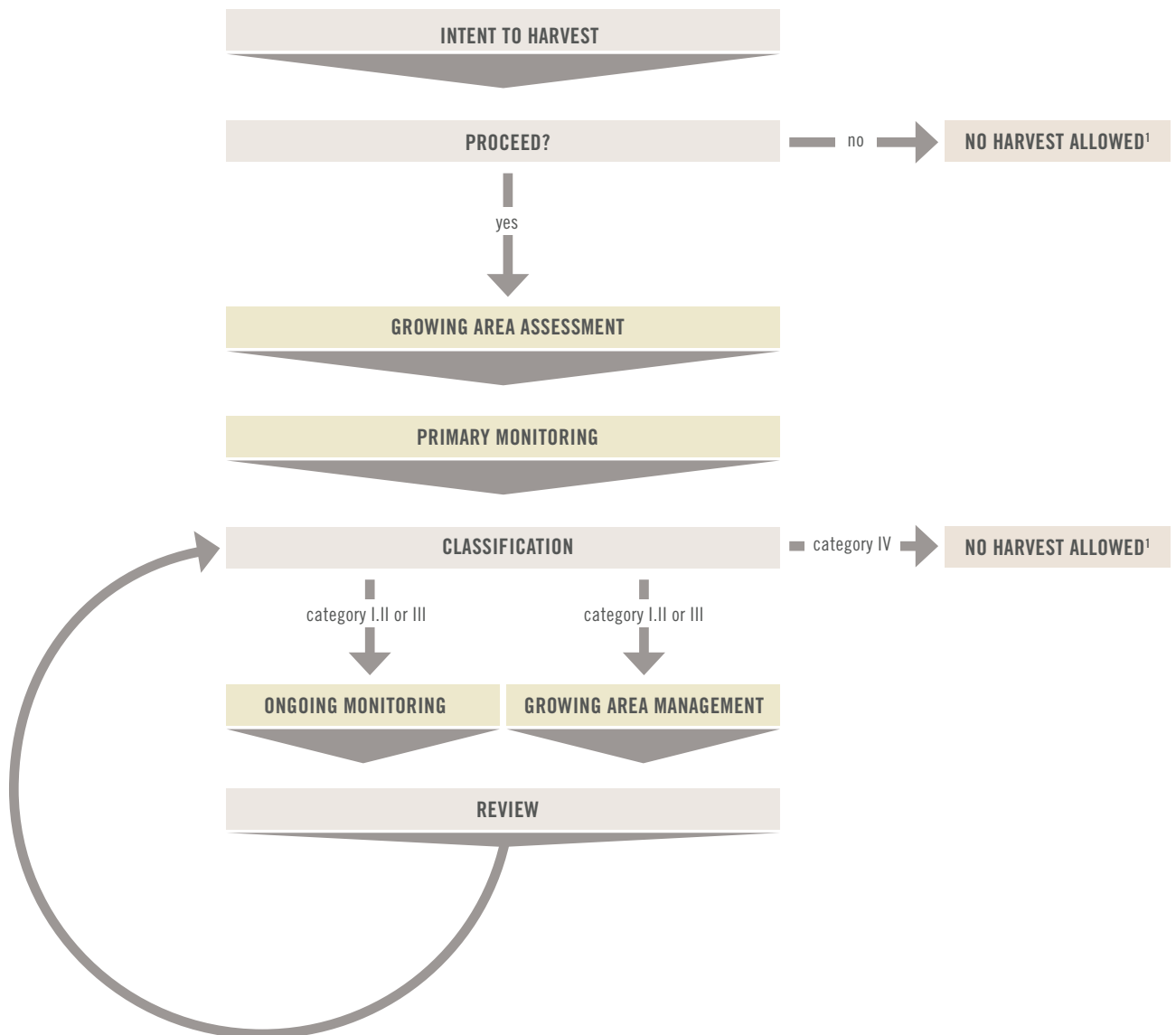
1 Some growing area management activities (e.g. surveillance) may be necessary for areas that are not subject to harvest.
  See Section 5.4 for classification categories.

TABLE 1.1   **RELATIONSHIP BETWEEN THE MAJOR SECTIONS OF THIS GUIDANCE DOCUMENT AND THE CODEX COP, AND EU AND US REGULATORY REQUIREMENTS AND GUIDANCE**

This table gives only the principal relationships and where compliance with EU and/or US criteria are required, the user should ensure that *all* relevant parts of the regulations and guidance are followed.

GUIDANCE DOCUMENT SECTION	CODEX COP FOR FISH AND FISHERY PRODUCTS	EU REGULATIONS AND GUIDANCE	U.S. REGULATIONS AND GUIDANCE[1]
Growing Area Risk Profile	Section 7.1	Not applicable[2]	Not applicable[2]
Growing Area Assessment	Section 7.2.1	Regulation (EC) 854/2004, Annex II, Chapter II, Section A.6 Community Guide[3], Sections 1 & 2 Good Practice Guide[5], Chapter 1	NSSP, Section II, Chapter IV, Part 01 NSSP, Section III, Chapter IV, Part 01 NSSP, Section IV, Chapter II, Part 07
Growing Area Monitoring	Section 7.2.2	Regulation (EC) 854/2004, Annex II, Chapter II, Section B Community Guide, Sections 4, 5 & 6 and Annex 1 Good Practice Guide, Chapters 3, 4, 5 & 6	NSSP, Section II, Chapter III and Chapter IV, Part 02 NSSP, Section III, Chapter III and Chapter IV, Part 02 NSSP, Section IV, Chapter II, Parts 11, 14 & 15
Growing Area Classification	Section 7.2.1	Regulation (EC) 854/2004, Annex II, Chapter II, Section A, as amended, and Annex II, Chapter II, Sections E & F Community Guide, Section 7 and Annex 1 Good Practice Guide, Chapter 7	NSSP, Section II, Chapter IV, Parts 02, 03 & 05 NSSP, Section III, Chapter IV, Parts 02, 03 & 05 NSSP, Section IV, Chapter II, Parts 01, 07, 16 , 18 & 19
Growing Area Management	Section 7.2.2	Regulation (EC) 854/2004, Annex II, Chapter II, Sections C, D, E & F[6] Community Guide, Section 7 Good Practice Guide, Chapter 7	NSSP Section II, Chapter II and Chapter VIII NSSP, Section III, Chapter II and Chapter VIII NSSP, Section IV, Chapter II, Parts 02, 03, 04, 05, 06, 08, 09, 12 & 13 and Chapters IV & V
Growing Area Review	Section 7.2.2	Community Guide Section 2.3.9 Good Practice Guide, Chapter 2, Section 2.14	NSSP, Section II, Chapter IV, Part 01.C NSSP, Section III, Chapter IV, Part 01.C NSSP, Section IV, Chapter II, Part 07

1. NSSP, 2015 revision. Section II contains the principal requirements, Section III public health reasons and explanations, and Section IV guidance documents;

2. No direct relationship but the Growing Area Risk Profile can be viewed as the first part of sanitary surveys (equivalent to the Growing Area Assessment in this Guidance);

3. European Commission, 2017;

4. EURL, 2017; 5. Regulation (EC) 854/2004, Annex II, Chapter III relates to official controls concerning *Pectinidae* harvested outside classified production areas.

CHAPTER 2
GROWING AREA
RISK PROFILE

The risk profile is the first stage prior to determining whether, in principle, a monitoring programme should be established for a growing area, and therefore an intention to classify and allow harvest. It comprises the acquisition, recording and assessment of available information related to the area. This will inform the further stages of the monitoring and classification process or, in certain circumstances show that the area is not suitable for harvest and therefore that no further resource should be allocated to the process. However, in the latter case, resource may still be utilized in order to assure that harvesting does not take place (see Section 6).

The level of detail gathered for the risk profile should be limited to that necessary for the initial assessment process. If more information or data than necessary for the process is supplied (e.g. full data on sewage discharges is provided by the environmental regulator), a summary should be presented in the risk profile and the fuller data set should be retained for use in the growing area assessment if a decision is taken to proceed with implementing a sanitation programme for the growing area.

If the sanitation programme is to be applied to recreational, as well as commercial, harvest, then the same process will need to be applied to all species that may be gathered in a growing area.

A growing area risk profile template is given in Annex 1.

2.1   AREA OVERVIEW

Recommendation

Briefly describe the geographical location and general nature (e.g. offshore, coastal, estuarine) of the growing area.

Explanation

The area overview provides general information relating to the growing area that will help readers and users of the growing area risk profile place other information provided later in the document into context.

2.2   SCOPE OF RISK PROFILE

Recommendation

Summarize the intended scope of the risk profile and any resulting sanitation programme for the growing area. The main considerations are whether each of the following are to be covered:

> Domestic (national) commercial sales;

> International trade (if so, define the existing or intended target regions of countries); or

> Recreational gathering[2].

Other considerations

It will also be relevant to consider whether, prior to preparation of the growing area risk profile, it has already been decided that a programme for domestic sales will be limited to one or more priority hazards or the range of hazards addressed will need to be constrained to meet the available resource.

Explanation

The scope defined within the risk profile will determine what regulations or other requirements may be relevant to the growing area sanitation programme. It will also affect other aspects of the risk profile and sanitation programme, such as the data that needs to be gathered (e.g. relating to the intended use, target consumers) and risk management activities for the growing area.

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[2]   The gathering of bivalve molluscs for consumption at home rather than for commercial sale.

2.3 EXISTING LEGAL FRAMEWORK

2.3.1 CURRENT RELEVANT FOOD SAFETY REGULATIONS, STANDARDS AND OTHER PROGRAMME REQUIREMENTS[3]

Recommendation

Define the national or other regulations, such as regional (e.g. European Union) or local regulations that is relevant to the sanitation programme for a growing area[4]. If a country has an interest in exporting products to a specific market, any specific import requirements of that market will be relevant, in addition to the regional, national and local legal requirements.

The relevant regulations (including amendments) should be listed together with a brief note of the any specific requirements of each item of regulation as they affect the growing area sanitation programme (assessment, monitoring, classification and risk management) of growing areas.

Some parts of food standards regulation (sometimes known as end product requirements) for bivalve molluscs may have a consequence for the growing area sanitation programme if specified levels of a hazard or indicator need to be assessed in the growing area, either in addition to, or instead of, in the final product. In such cases, this regulation will need to be listed.

Other considerations

For recreational gathering or product to be traded within the country of production, national and local regulations may apply. For product to be traded internationally, the importing country or countries may have their own import requirements that are also relevant for production purposes. It may be the case that exported product will also have to comply with national (and local) requirements prior to export as well as the requirements stipulated by the importing country.

Regulations providing the food safety requirements for bivalve molluscs may be included in more than one legal instrument. For example, general food law may introduce the principle that all food must be safe and free from harmful substances or pathogens. While other regulation may contain the specific food safety requirements relating to the production of bivalve molluscs.

Regulations followed for trade or domestic purposes may contain a requirement specified under general food law for product to be free from harmful substances or pathogens. In such circumstances, those requirements need to be considered in addition to the bivalve mollusc-specific regulations.

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[3] Other programme requirements may be given in official desk instructions or protocols which give further detail on the procedures to be followed to implement the regulatory requirements.

[4] If all or part of a growing area sanitation program are to be developed prior to the establishment of relevant national and/or local regulation, the framework for the programme will need to be defined.

The consequences for the growing area risk profile, and the rest of the growing area sanitation programme, will need to be considered if there are subsequent changes in the relevant regulations or if additional regulation needs to be addressed through an expansion of international trade destinations.

It may also be useful to list items of regulations that apply to the food safety of bivalve molluscs from harvest onwards. In order to simplify the regulations section of the growing area risk profile, this may be best done as an annex to the document.

Explanation

Existing regional, national and/or local regulations that must be met, either for domestic or international trade, will dictate specific elements of a bivalve sanitation programme, and any associated standards, which must be met. It is essential that such specific aspects are addressed within the framework presented in this guidance. The existing regulatory requirements will also have an implication for the resourcing of the programme for the growing area.

2.3.2   JURISDICTIONS, RESPONSIBLE AUTHORITIES

Recommendation

Identify and record which official body(ies) is(are) responsible for the application of the sanitation programme and associated monitoring and enforcement activities.

Other considerations

Responsibility within national programmes may be divided between a number of national authorities. For example, there may be a separation between primary responsibility for policy and implementation of the overall programme, growing area monitoring activities and growing area enforcement. Divisions in responsibility may be between different bodies at the national level (e.g. Ministries) or between one or more national bodies and local authorities. Where such divisions of responsibility occur, it is not only important to identify them but also to determine how they will affect the programme at the specific growing area level (responsible local offices, contact details, etc.).

Explanation

It is important to be clear as to which official bodies are primarily responsible for the application of a sanitation programme for a growing area and whether any other bodies also have responsibilities under the programme. In addition, in many countries, responsibility for the application of food safety regulations is split between national, regional and/or local authorities. Again, it is important to document the separate responsibilities and any implications for the programme at the specific growing area level.

2.3.3 OTHER OFFICIAL BODIES WITH RESPONSIBILITIES RELATING TO GROWING AREAS

Recommendation

Identify and record which official body(ies) have responsibilities for the application and/or enforcement of other regulations relating to aquaculture and/or wild harvest of bivalve molluscs and environmental quality. These include the following aspects:

> *Environmental quality:* control of discharges (sewage and industrial), disposal of waste to the marine or estuarine environment, water quality protection and monitoring (including for recreational water activities);

> *Animal health regulations* (relating to both terrestrial and aquatic animals, including biosecurity implications). Associated consequences may be restrictions on aquaculture or harvesting practices, access for shoreline surveys and access for sampling;

> *Controlled and protected areas:* There may be statutory controls on placing equipment (including that associated with aquaculture) in the marine environment, or on activities,

including aquaculture and harvest that can be undertaken. Some countries protect areas and recognise the rights of indigenous people, or for cultural reasons, limit the harvest of natural resources. It is sensible for the authorities responsible for the growing area sanitation programme to ensure that the aquaculture or wild harvest activities can be legally undertaken in a growing area before expending resource towards classification and monitoring;

> *Fisheries regulations:* Aquaculture leases may contain specific provisions that are relevant to the growing area assessment and the subsequent programme. Wild fisheries may be subject to harvest controls (e.g. by season for conservation purposes) that be relevant to sampling plans and growing area enforcement. In additional, sanitation programme authorities (or their agents) may need to obtain approval from the fisheries authorities to take bivalve samples during closed periods.

Explanation

Liaison with other official bodies is beneficial (or may even be required) in order to:

> obtain information relevant to the development of the risk profile and growing area assessment;

> ensure that the sanitation programme is conducted in accord with this other regulations (e.g. constraints on sampling);

> obtain information related to expected or unexpected events for growing area management activities; and

> support collaborative enforcement activities.

2.3.4 INTERACTIONS BETWEEN FOOD SAFETY AUTHORITIES AND OTHER RESPONSIBLE BODIES

Recommendation

Arrangements for collaboration with other bodies that will contribute significantly to the sanitation programme for the growing area should be formalized. For other bodies that will make a minor contribution to the sanitation programme, the arrangement may be formal or informal. Formal arrangements may already be specified in regulations but should otherwise should be established through memoranda of understanding, specific agreements or letters of intent.

For each body, the roles and responsibilities should be documented together with the practical arrangements: i.e. the part(s) of the programme to which the interaction is relevant and practical details such as the local offices and contacts.

Other considerations

The other responsible bodies will be those with powers outlined in Section 2.1.3. In addition, there may be non-governmental organizations, industry co-operatives or private companies that may also have a role in assisting effective implementation of a bivalve mollusc sanitation programme. The nature of bodies with the same function may vary between or within countries. For example, ownership of, and the running of, sewage treatment works may be by national, regional or local authorities or by private companies.

Explanation

Interaction and cooperation with other bodies, official or otherwise, will contribute significantly to the effective operation of a bivalve mollusc sanitation programme. It is therefore necessary to establish the means by which the interaction will be undertaken and to document this so that all involved with the sanitation programme, and those in the other bodies, are clear as to the roles and responsibilities which apply.

2.4 CURRENT INDUSTRY SITUATION/CURRENT RESOURCES/AVAILABLE RESOURCES

2.4.1 SPECIES OF BIVALVE MOLLUSCS TO BE HARVESTED

Recommendation

All species of bivalve mollusc that are, or are intended to be harvested, from a growing area should be identified. Where appropriate, other information covered by Sections 2.4.2 and 2.4.3 will need to be recorded separately for each species.

Other considerations

Although an area may be identified for the harvest of one or more species of bivalve, it may be that other species are obtained as bycatch. If these are allowed to go for consumption, they should also be identified for inclusion in the sanitation programme.

Section 1.2 identified that this guidance does not address the monitoring and control of diseases of bivalve molluscs. However, it would be appropriate at the Growing Area Risk Profile stage to determine whether endemic bivalve diseases are an issue for the proposed harvested species. This may have an influence on international trade and /or the method of post-harvest treatment that is needed. There is also the possibility that disease may markedly reduce the bivalve stock(s) in the proposed growing area, making the area economically unviable and thus affecting the cost/benefit analysis for the sanitation programme (Section 2.11).

Explanation

The species of bivalve may need to be defined in order to undertake formal actions for both classification and enforcement purposes (e.g. if the area is classified for one or more specific species of bivalves). In addition, differences in the biology and physical location of bivalve species will have implications for the determination of relevant hazards, the growing area assessment and subsequent monitoring, management and enforcement activities.

2.4.2 LOCATION OF BIVALVE MOLLUSC RESOURCE(S)

Recommendation

The location and extent of the occurrence of each species of bivalve mollusc to be addressed under the programme should be determined. The area of current and/or intended harvest should also be determined as this may not be as extensive as the area covered by the species.

Other considerations

The occurrence of a bivalve resource over a larger area than that intended for harvest usually applies to wild harvest. However, a growing area used for aquaculture may have some parts that are used for harvest for consumption while other parts may be used for production of juvenile stock that will be on-grown elsewhere.

Bivalves may also be grown in artificial ponds or land-based tanks fed with seawater. These will need to be addressed as growing areas within the sanitation programme. However, assessment of likely pollution sources is likely to be simpler than for a growing area in the marine environment and monitoring options will be more constrained.

Explanation

The location of the resource of each bivalve species is important in assessing potential hazards together with sources of contamination and their potential impact. For programmes that include monitoring of indicators and/or hazards in bivalve molluscs, it is necessary to know where samples may be obtained. Effective risk management actions, including surveillance, also require knowledge of the location and extent of the resource(s).

2.4.3   CULTIVATION AND HARVEST PRACTICES

Recommendation

The information specified in Sections 2.4.3.1 to 2.4.3.8 should be obtained and recorded for each species that is to be assessed for inclusion in the sanitation programme for the growing area.

Other considerations

Relevant information on seedstock production in the area may be included in order to ensure that any implications for a sanitation programme can be assessed.

Explanation

The information is required in order to properly inform the Growing Area Assessment and subsequent Growing Area Monitoring activities and/or the Growing Area Management.

2.4.3.1 Type of cultivation and harvest

Recommendation

Identify whether the current or intended gathering is wild harvest, ranching[5] or aquaculture. If appropriate to the programme, determine the presence and significance of recreational harvest.

Explanation

The type of aquaculture operation, where relevant, and type of harvest will influence the ability to operate management controls for conditional classifications and also management options with respect to contaminants (e.g. pathogens, biotoxins, or chemical contaminants). In addition, the type of operation may affect the ability to obtain samples of bivalve molluscs from sites within the growing area.

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[5]   Ranching is the extensive cultivation of bivalve molluscs, or augmentation of wild stocks in their natural environmental niche.

2.4.3.2 **Location in the water column**

Recommendation

Identify and record where the bivalve mollusc resource will be located in the water column – in the sediment, on the seabed, on rocks, or on natural or artificial structures, or in/on aquaculture equipment. Also identify if the resource will be exposed at certain states of tide.

Explanation

Depending on species, bivalve molluscs may naturally grow in sediment, on the seabed, or attached to rocks and other structures (natural or artificial). Ranching comprises augmentation of natural stocks of bivalve molluscs and therefore growth will take place in the same location as natural stock. Aquaculture may take place in the natural location for the species but will often be undertaken using methods that aid maintenance operations and eventual harvest and that may also increase the density over that which would be obtained during natural growth. Such methods may include placement in bags, nets, or the use of suspended ropes (often from long-lines or rafts). Bags may be placed on the seabed but will more usually be placed on trestles or suspended on lines.

The location of the bivalve molluscs within the water column may, depending on the area, influence the types of contaminants to which the animals are exposed, the concentration of those contaminants, and the uptake and depuration of the contaminants by the bivalves. This will influence the initial assessment and subsequent monitoring. This may also have an implication for any associated sampling programme.

2.4.3.3 **Harvesting methods**

Recommendation

Identify and record the intended method(s) of harvest.

Other considerations

Commercial harvesting methods may be mechanical or manual (hand-picking or by diving) whereas recreational harvesting methods will usually be manual. Mechanical harvesting methods for bivalve molluscs include dredging (conventional towed, or hydraulic), mechanical stripping of lines or bouchots and mechanical lifting of lines and nets.

Explanation

The harvest method may be relevant to the assessment process from the consideration of additional exposure of the bivalve molluscs to contaminants, shock to the bivalve molluscs, and implications for sampling if the monitoring programme is to be based on, or include, sampling of the bivalves themselves.

2.4.3.4 **Relaying, conditioning or wet storage activities**

Recommendation

Identify and record whether there are current relaying[6], conditioning[7] or wet storage[8] activities undertaken in the area, or whether there is the intention for these to be undertaken.

Other considerations

The conduct, or intended conduct, of such activities, and the location(s) of these, should be included in the assessment process and any subsequent monitoring and surveillance. Regulations may require such activities to be undertaken in separately designated (and potentially classified) areas that are geographically distinct from growing areas using for primary harvesting.

Bivalves may be moved from coastal locations to deep water holding sites for specific purposes, e.g. to promote reduction in the concentration of pathogenic vibrios.

Relaying, conditioning and wet storage may also be conducted in artificial ponds or land-based tanks fed with seawater.

Explanation

Relaying, conditioning and wet storage activities occur prior to final harvest and therefore knowledge of these is essential to the overall assessment and management of an area. Additional contamination of initially harvested product may occur during these activities if the water quality is adequate and if the operation is not managed properly. Relaying is an explicit process consequent to classification and needs to be properly controlled if it is to achieve its objective.

2.4.3.5 **Distance to landing sites from growing areas**

Recommendation

Determine and record the distance from the growing area to any defined or approved landing sites. For growing areas harvested by hand, determine and record the distance from the actual collection areas to any defined or approved base.

Other considerations

Where multiple harvesting operations are undertaken at different locations prior to landing, these, and the overall time involved, should also be recorded.

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[6] Relaying is the removal of bivalve molluscs from a contaminated growing area to an acceptable growing or holding area under the supervision of the responsible authority and holding them there for the time necessary for the reduction of contamination to an acceptable level for human consumption.

[7] Conditioning is the act of placing live bivalve molluscs in tanks, floats or natural sites to remove sand, mud or slime and improve product acceptability.

[8] Wet storage is the temporary storage of bivalve molluscs after harvest from a classified area and prior to sale or processing. It is not intended to reduce microbiological contamination.

Explanation

The distance will affect the minimum time that the harvested bivalves will be out of the water prior to any land-based operations such as bulk packing and/or transport. There will therefore be the potential for the exposure of bivalve molluscs to temperature abuse. Other aspects that will influence the time between harvest and landing are the duration of an individual harvesting operation and whether the harvesting vessel will undertake multiple harvesting operations.

2.4.3.6 Industry capability

Recommendation

For commercial harvesting, consider whether the industry has the resources to bring plans for utilization of a new area to fruition. Also determine whether the local industry is only involved in the harvesting process or whether the same businesses are involved in subsequent transportation and/or processing (e.g. depuration, commercial cooking).

Explanation

Whether or not the industry has enough resource is one element that needs to be considered in the cost/benefit analysis (see Section 2.11). Whether the same businesses are involved in more than one step of the harvesting, transport and processing of the bivalves is a consideration in the application of growing area management procedures (see Section 6).

2.4.3.7 Seasonality of harvest

Recommendation

Determine whether the intended harvest period is to be restricted to, or concentrated on, part of the year.

Other considerations

The harvest period may be restricted to a certain time of year by a traditional season for the consumption of a species, patterns of commercial activity (perhaps with respect to other fisheries) or closure period(s) imposed for the purpose of stock conservation.

Explanation

A seasonal pattern to the harvest may be relevant to an assessment of the likely importance of specific hazards (e.g. norovirus, vibrios, biotoxins, some chemical contaminants) and any monitoring that may be considered for these. In addition, if harvest is only to be undertaken for a restricted part of the year, it may be determined that monitoring outside these periods may not be warranted.

2.4.3.8 Growing Area production capability

Recommendation

Where possible, the harvestable biomass present within an area and/or the production capability of the area should be estimated. If the area is currently harvested, this should be compared with estimates or records of recent harvested amounts.

Other considerations

These estimates may need to be determined separately for commercial and recreational harvests. An estimate can then be determined for the value, at first sale, of the present and future yield from the area. It will also be useful to estimate, where possible, of the potential for change in the extent of use of the area. This might be the possibility for expansion, with an increase in the extent of aquaculture or the use of ranching to enhance wild stocks. Alternatively, there could be a likely decrease due to exhaustion of commercially-sized bivalves by overfishing.

Explanation

This information may be used in the evaluation of the cost and benefit of establishing a sanitation programme for the growing area.

2.4.4 SEASONAL WATER AND AIR TEMPERATURES

Recommendation

Determine and record general patterns of water and air temperatures through the year and variation between years.

Explanation

These are most relevant to considerations relating to the risk from naturally occurring pathogenic vibrios (see Section 3.1.2.5). However, there are other considerations: in some parts of the world, norovirus risk from bivalve consumption is much higher during the colder months. Seawater temperatures are also relevant to the kinetics of the natural depuration of contaminants.

2.5   EXTENT OF THE ASSESSMENT AREA[9]

Recommendation

The extent of the assessment area should be determined relative to the location and extent of the intended harvesting area together with the location and extent of the surrounding catchment(s) and likely contaminant transport distances, and dilution and dispersion in the marine environment.

Other considerations

The extent of the assessment area may need to be revised during the conduct of the risk profile and subsequent growing area assessment (or even at a subsequent review) in response to additional information and/or data that is received during these processes.

Explanation

Definition of the extent of the assessment area is essential as this determines the area for which information and data needs to be gathered for both the risk profile and the growing area assessment. The assessment area needs to be larger than the area of intended harvest as it needs to include any pollution sources that may impact on the subsequently defined growing area.

The pollution sources that may be relevant to an assessment will be dependent on the transport of contaminants via watercourses[10] and currents.

2.6   EPIDEMIOLOGICAL AND PUBLIC HEALTH DATA

Recommendation

Identify and record local, regional, national and international epidemiological data relevant to the bivalve species of interest.

Other considerations

The use of epidemiological data in identifying and potentially ranking hazards requires that there be a robust system for recognizing and recording food-borne illness, procedures for assessing which foods consumed prior to an event were most likely to have contributed to the illness, and a robust system for tracing potentially implicated foods back through the key points in the food chain to the primary source (the growing area with respect to bivalve molluscs).

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[9]   The area to be covered by the Growing Area Assessment – based on the extent of the fisheries, the catchment area(s) potentially impacting on the growing area and the estimated pollutant transport distance in the marine/estuarine environment.

[10]   A watercourse is natural or artificial channel through which water flows: the term includes rivers, creeks, streams and canals, including those running through culverts or underground.

Epidemiological data for use within the risk profile may be obtained from scientific publications, epidemiological bulletins, the outcomes of official reviews of illness associated with bivalve mollusc consumption and official investigations into specific outbreaks. This may be supplemented with local knowledge. However, the strength of association may need to be considered when determining the significance that will be given to the hazard within the risk profile.

There are several levels at which epidemiological information can be applied to the risk profile:

> international data on illnesses associated with the consumption of bivalve molluscs, including that which applies to the species of molluscs relevant to the growing area under consideration. This may be derived from epidemiological bulletins and/or the scientific literature;

> regional or national or data relating to:

> the occurrence of illnesses in the population due to hazards identified at the international level (i.e. confirming that those hazards occur in the country and may be of relevance with regard to bivalve mollusc consumption);

> specific association of illnesses with consumption of bivalve molluscs, particularly where this relates to the species relevant to the growing area under consideration;

> Growing area-specific data;

> this may relate to the prevalence and/or incidence of relevant illnesses in the local community; or,

> if the area is being, or has previously been used for bivalve mollusc harvest, evidence for outbreaks or incidents of illness linked to bivalve molluscs harvested from the area.

In general, this will be addressed in the sequence: "International data" > "Regional or national data" > "Growing area-specific data". The absence of good relevant epidemiological data at the regional, national or local level does not necessarily mean an absence of risk. The underlying supposition needs to be that, if there is clinical data indicating that one or more hazards is actually causing illness in the population, the hazard will need to be addressed within the bivalve mollusc sanitation programme.

The investigation of outbreaks/illnesses possibly associated with the consumption of bivalves from a growing area may take the form of a root cause investigation. There are a number of steps in such an investigation:

1. what was the event?

2. Data collection.

   > What happened within the event and was the sequence of those occurrences?

3. Identification of causal factors. What was the sequence of the occurrences leading up to the event, what were the possible contributing factors?

4. Root cause identification.

    > Was there a main underlying factor, why did it occur?

5. Recommendation generation and implementation.

    > How to prevent the event occurring again and how to implement the solution(s).

Studies on groups of outbreaks related to a single commodity, or group of commodities, may be undertaken at the international, national or regional level in order to determine whether any common root causes are involved and thus whether any common solutions might be applied (e.g. see Hay, McCoubrey and Zammit (2013)) .

Once the available evidence has been reviewed and documented for the risk profile, any gaps (e.g. lack of appropriate outbreak data, inability to identify root causes) should be identified together with potential means by which these may be addressed either within the sanitation programme or through other agencies.

It may be that a country or specific area is already subject to some form of bivalve mollusc sanitation programme, and the risk profile is being undertaken to extend the programme to cover other requirements (e.g. an additional trade destination). In such a case, the epidemiological data for the country and/or area needs to be evaluated in the context that the existing programme may be mitigating some or all of the risks relating to one or more hazards.

Changes in the species, type or extent of bivalve production, or harvesting season, may change the risk from that which previously applied.

Explanation

Epidemiological evidence is the best indication that a hazard needs to be considered within a sanitation programme. Lack of specific evidence relevant to the local programme, either specific to bivalve molluscs, or to the area in question, may mean that the use of more general data is relevant, e.g. that from international or national sources. In addition, many hazards may affect more than one growing area, and thus part of the assessment process is to determine whether, for a specific growing area, data on outbreaks from other nearby growing areas may be directly relevant.

2.7 INTENDED USE OF PRODUCTS AND CONSUMING POPULATION

This information is a key part of the risk profile. However, the ability to gather relevant data tends to become more difficult the further the distance between location of final sale and consumption is from the growing area. In addition, consumption patterns, preparation methods and type and proportion of at-risk groups differ between and within countries and therefore trade to multiple locations complicates identification of the key characteristics to consider in this section. In general, where information is lacking or there are known differences, worst case assumptions on a case-by-case basis could be considered in the assessment process.

2.7.1 SOCIETAL CONSUMPTION PATTERNS

Recommendation

Obtain and record information on the frequency and quantity of bivalve mollusc consumption for each species under consideration for the growing area.

Other considerations

The likely consuming population should be taken into consideration in determining the level of detail of consumption data that is relevant.

Guidance on the conduct of food consumption studies can be found in FAO (2009).

Explanation

Consumption patterns are relevant to estimation of exposure to hazards, including chronic exposure to chemical contaminants such as heavy metals. The patterns may vary markedly between bivalve species as well as between regions and countries, gender, age group and societal group.

2.7.2 METHOD OF PRESENTATION, PROCESSING AND/OR PREPARATION

Recommendation

The intended form in which the harvested bivalves are to be marketed and/or consumed should be determined.

Other considerations

Full details of the final processing to be applied may not be available if the harvested product is to be marketed outside the immediate jurisdiction of the authority in charge of the growing area. This will especially be the case if the product is to be exported live. At this stage it may only be possible to determine whether the intent is to market the product raw or after some form of post-harvest processing.

Explanation

The form in which the bivalves are intended to be marketed (e.g. whole, eviscerated) will affect the hazards which may be relevant to the general consuming population or at-risk groups. Regulations may require bivalve growing areas used for commercially cooked or canned product to be subject to the same assessment, monitoring, classification and management procedures as those used for live or raw product. In such a situation, the cooking or canning process is relevant to the hazard assessment but may not affect the subsequent procedures dictated by the regulations.

The assessment based on intended form of presentation may be markedly affected by the outcome of the assessment and classification process which may dictate that the level of processing be markedly different to that which was originally intended.

2.7.2.1 **Live or Raw (unprocessed)**

Recommendation

Determine whether the product is to be sold live, on the half-shell or shucked. If shucked determine whether the digestive track and/or other parts of the bivalve have been removed before sale or consumption. Also determine whether any parts are to be used separately for human consumption (e.g. scallop gonads). Record the appropriate information.

Explanation

The state of the raw bivalve at consumption will affect the degree of risk posed by some identified hazards.

2.7.2.2 **Post-harvest processed**

Recommendation

Identify and record the intended method of post-harvest processing and the parameters (e.g. temperature and time, where relevant) that will be used. The processing methods may include depuration[11], short-term relaying[12], long-term relaying[13], cooking or partial cooking (commercial or domestic), High Pressure treatment, pasteurization and freezing (including Individually Quick Frozen (IQF)).

Explanation

These details will enable consideration as to whether some potential hazards may pose a risk to final consumers. However, processing undertaken in the absence of a programme requirement (e.g. depuration of bivalves from a Category I area) may or may not be deemed to provide sufficient consumer protection (e.g. some harvested product may be traded elsewhere on occasions) and the classification status that is determined by the relevant authority may need to either formally require such processing or may necessitate additional processing to that originally intended by the industry.

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[11] The reduction of micro-organisms 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 seawater suitable for the process, which may be treated or untreated.

[12] Short-term relaying is undertaken as an alternative to depuration and is intended to reduce the content of pathogenic bacteria, such as *Salmonella enterica*, to acceptable levels. It is usually undertaken for periods up to one week.

[13] Long-term relaying is intended to reduce the content of pathogenic viruses, such as norovirus, to acceptable levels. It is usually undertaken for periods between two weeks and two months, dependent on bivalve species and water temperature.

2.7.3 HIGH RISK CONSUMERS

Recommendation

Define the consuming sub-population in terms of age range and any known at-risk groups (immunocompromised, underlying illness such as liver disease, etc.).

Other considerations

The consuming sub-population characteristics may vary by locality, region or country. There may therefore be differences for the same product intended to be sold in different parts of the same country, or to be traded to different countries.

Explanation

Very young and very old people usually show greater susceptibility to many pathogens, or are prone to more severe illness when infected, due to their immune systems being less effective than those of otherwise healthy young adults. People of any age may show greater susceptibility if the capability of their immune system is compromised by genetic disorders, underlying illness such as liver disease, infection with human immunodeficiency virus, some types of neoplasia ("cancers"), injury or removal of the spleen and some types of therapy such as chemotherapy. In addition, specific potentiation of infection may be seen with some underlying illnesses: for example, growth of some pathogens in the body, e.g. *Vibrio vulnificus*, is potentiated by conditions that result in iron overload in the body.

2.8 OTHER RELEVANT INFORMATION

Recommendation

Summary information on the following features of the assessment area should be assembled and recorded:

> Aspects related to contamination sources:

   > land-based human activity - (population, tourist activity, industrial activity, mining, transportation) that may produce pollutants;

   > water-based human activity (ports, marinas, concentrations of boating activity);

   > sewage discharges (location of continuous and intermittent sewage discharges together with the intended level of any sewage treatment) locations of potential collection system discharge points (e.g. sewage pumping (lift) stations, combined sewer overflows) as well as underground sewage disposal systems;

   > areas with high concentrations of farm animals;

   > areas with high concentrations of wild animals and birds;

   > watercourses;

   > geology (with respect to naturally occurring chemical contaminants).

> Aspects related to the impact of hazards (including biotoxins and naturally occurring marine vibrios):

  > topography;

  > bathymetry and hydrodynamics;

  > hydrology (including seasonal changes in freshwater inputs and their influences on the receiving waters);

  > meteorology (including precipitation patterns and distribution and prevailing winds with season);

  > seawater temperature and salinity; and

  > existing monitoring data relevant to microbiological, biotoxin and chemical hazards (may relate to previous or existing growing area monitoring or other programmes such as marine water quality, sediment contaminants, etc.).

> Other available data deemed relevant to the characteristics of the assessment area and the likely hazards identified in Section 2.6.

> If *V. parahaemolyticus* and/or *V. vulnificus* have been identified as likely hazards, information/data should be sought as to whether the organism will multiply in the bivalve species to be harvested.

Other considerations

Much of this information may be obtained from existing maps, charts and from other relevant authorities such as agriculture, environmental and fisheries regulators. The purpose of gathering such information is given in Sections 3.1 and 3.2. It must be emphasized that it is intended that summary information be gathered to inform the risk profile and that acquisition of detailed information and data should only be undertaken for the growing area assessment if the recommendation from the risk profile is to proceed to that stage.

Explanation

Summary information on sources of contamination and their actual or potential impact, in combination with the assessment of the likely hazards, provides the basis for a cost benefit analysis and also whether an area is likely to be so affected by a hazard that harvesting should not be permitted, regardless of the cost benefit outcome. Although similar types of information may be acquired for both the Growing Area Risk Profile and the Growing Area Assessment, the level of detail needed for the Growing Area Risk Profile is usually much less and individual data sets are usually not required.

2.9   HAZARDS TO BE CONSIDERED

Recommendation

Define the hazards that may be relevant to the intended area of harvest on the basis of the information and data gathered to meet the guidance recommendations of Sections 2.2 to 2.8 inclusive.

It is important that all relevant specific hazards are identified (e.g. all specific pathogens with respect to microbiological hazards, and all compounds with respect to chemical contaminants) as the individual characteristics of each hazard may be relevant to the assessment, monitoring and management procedures.

Where only parts of a bivalve will be marketed for human consumption (e.g. scallop adductor muscle) the assessment process should address those hazards relevant to that (those) part(s).

Other considerations

The hazards may include microbiological, chemical hazards, radiological hazards and marine biotoxins. References CAC (2014) and FAO (2004) should be consulted with respect to chemical hazards and biotoxins. FDA (2015) gives guidance on "Action Levels, Tolerances and Guidance Levels for Poisonous or Deleterious Substances in Seafood" and specifies levels relating to the U.S. NSSP. These do not include levels for heavy metals. Basic principles underpinning EU regulation on contaminants in food are given in Council Regulation 315/93/EEC (EC, 1993) and maximum levels for certain contaminants in food in the EU are given in Commission Regulation (EC) No 1881/2006 (EC, 2006a, as amended).

With respect to microbiological pathogens, the hazards that may be relevant include those in Table 2.1. Where the evidence of bivalve-associated infection is given as "No", no specific epidemiological association has been shown but the presence of the pathogen has either been demonstrated in bivalves or is considered to be extremely likely. Apart from marine vibrios (including *V. parahaemolyticus* and *V. vulnificus)*, the listed microbiological hazards are generally associated with human and/or animal faecal contamination. The likelihood of significance with respect to an individual growing area may be determined from the predominant sources of contamination identified in the assessment area (see Sections 2.8 and 3.1). However, toxigenic *V. cholerae* (including O1 and O139) do occur in the marine and estuarine environment and some strains of *Salmonella* spp. have been shown to at least survive for extended periods in such locations.

The list provides a suggestion of those microbiological hazards that may need to be considered. Not all of these potential hazards will be relevant to all countries or growing areas. There may be additional hazards that are applicable in the local situation. New hazards may be identified over time and these should also be taken into account.

Some hazards may need to be addressed due to regulatory requirements, irrespective of relevance or importance at the growing area level. Where regulatory requirements do not apply, different hazards may potentially be assigned a relative risk in order to prioritise application of programme resource. Criteria that may be used in the prioritization process include the potential number of illnesses associated with a specific hazard and the severity of the illness (which may differ between parts of the population). Information and data on epidemiology (Section 2.6) and intended use and consuming population (Section 2.7) will be relevant to a prioritization process. The additional information or data gathered in support of the recommendations in Section 2.8 is relevant to the consideration of appropriate hazards in relation to the types of hazards that may arise from faecal or industrial inputs and the effect of environmental factors (e.g. water temperature and salinity with respect to potentially pathogenic marine vibrio species).

Explanation

The hazards relevant to an area will determine whether any additional monitoring is required in addition to any base monitoring that is required by relevant regulations and also will determine growing area management, including any additional processing requirements that may be required (e.g. if vibrios are considered to be a significant hazard) and any specific expected event management plans that may be necessary.

TABLE 2.1   **PATHOGEN MATRIX**

HAZARD RANKING	PATHOGEN	EVIDENCE OF BIVALVE-ASSOCIATED ILLNESS	FREQUENCY OF BIVALVE-ASSOCIATED ILLNESS[1]	SEVERITY OF ILLNESS[2]	LIKELY SOURCES OF CONTAMINATION TO THE AQUATIC ENVIRONMENT
Primary hazards	Norovirus (also Sapovirus and Aichi virus)	Y	Common[4]	Usually not severe	Human faeces
	Hepatitis A virus	Y	Moderately common in endemic areas[4]	Moderately severe[5]	Human faeces
	Salmonella typhi and *Salmonella paratyphi*	Y	Moderately common in endemic areas	Severe	Human faeces
	Other *Salmonella* serotypes	Y	Moderately common	Usually not severe	Human or animal[3] faeces
	Vibrio parahaemolyticus	Y	Moderately common	Usually not severe	Autochthonous
	Vibrio vulnificus	Y	Rare	Severe	Autochthonous

HAZARD RANKING	PATHOGEN	EVIDENCE OF BIVALVE-ASSOCIATED ILLNESS	FREQUENCY OF BIVALVE-ASSOCIATED ILLNESS[1]	SEVERITY OF ILLNESS[2]	LIKELY SOURCES OF CONTAMINATION TO THE AQUATIC ENVIRONMENT
Secondary hazards	Toxigenic *Vibrio cholerae*	Y	Moderately common in endemic areas	Severe without proper medical support	Human faeces, sometimes autochthonous
	Other *Vibrio* species	Y	Moderately common	Usually not severe	Autochthonous
	Campylobacter spp.	Y	Rare	Usually not severe	Human or animal faeces
	Listeria monocytogenes	Y (smoked bivalves)	Rare	Usually not severe[5,6]	Animal faeces
	Giardia intestinalis	Y	Rare	Usually not severe[5]	Human or animal faeces
Potential hazards	Hepatitis E virus	N	N/A[7]	Moderately severe[5]	Animal faeces (pigs, wild boar, deer, rats)
	Yersinia enterocolitica	N	N/A	Usually not severe	Animal faeces
	Microsporidia	N	N/A	Usually not severe[5]	Animal faeces
	Cryptosporidium parvum	N	N/A	Usually not severe[5]	Human or animal faeces
	Toxoplasma gondii	N	N/A	Usually not severe[5,8]	Animal faeces (primarily cats)

1. Frequency expected in the absence of an appropriate sanitation programme. The frequency will vary markedly by continent, country and even region. It may also change with time.

2. Expected severity in immunocompetent persons without other underlying disease. This does not apply to *V. vulnificus*, which usually only causes symptomatic illness in persons who are immunocompromised or have underlying illness.

3. The term animal is used to cover mammals and birds. However, some enteric pathogens (e.g. some *Salmonella enterica* serovars) can be carried by cold-blooded animals such as reptiles.

4. The frequency of norovirus and hepatitis A infection will only be partly reduced by a programme based on faecal indicator bacteria.

5. These pathogens may produce severe and/or chronic illness in some patients.

6. May be severe in immunocompromised patients and may have severe consequences for the foetus if a pregnant woman is infected.

7. N/A = Not applicable.

8. *Toxoplasma gondii* can result in severe consequences for the unborn baby if infection occurs in a pregnant woman.

2.10 PROGRAMME CAPABILITIES AND CAPACITIES

2.10.1 GENERAL

Recommendation

Determine whether the responsible authority and other organizations that will be formally involved in implementing the growing area sanitation programme have the capability and capacity to undertake the recommended activities covered by Sections 3 to 7 of this guidance. Identify any gaps or constraints.

Other considerations

The items that may need to be taken into account include:

> Relevant authority (determined by regulations, programme protocols, delegation, etc.);

> Appropriate budgetary resource;

> Sufficient appropriately qualified staff;

> Ability to provide appropriate training for staff; and

> Relevant and sufficient equipment, computers, software.

Explanation

An effective sanitation programme for a growing area requires the official bodies and other organizations having responsibility for undertaking the various parts of the programme to have the relevant authority and the appropriate resources to perform their identified tasks.

This relates to all parts of the programme. For example, there is no point in the growing area assessment, monitoring and classification activities if appropriate risk management activities cannot be applied effectively.

2.10.2 LABORATORY

Recommendation

Determine the availability of one or more laboratories, and their capacity, with respect to potential analyses relating to the hazards identified in Section 2.9.

Other considerations

Such analyses may be for indicators (e.g. faecal indicator bacteria, MSC[14] and/or potentially toxic phytoplankton) or the hazards themselves (pathogens, biotoxins, chemical contaminants and/or radionuclides). The laboratories should be competent to undertake the analyses; should be accredited to ISO 17025 (or an equivalent standard); and should take part in appropriate comparative testing (proficiency testing or external quality assessment). There is a need to consider the location of suitable laboratories in relation to the growing area, with the key factors being the time taken for samples to reach the laboratory (with respect to any criteria on maximum delay between sampling and start of analysis; see Section 4.1.5) and the cost of transport.

A wider range of sample types and anticipated concentration of hazard or indicator may need to be analysed for samples from shoreline surveys than from routine monitoring and it is important that the laboratory(ies) intended to be use have

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[14] In this sense restricted to F(+)RNA coliphage – positive sense single-stranded RNA bacteriophages that infect *E. coli* via adhesion to the F-pili.

the capability and capacity to analyse those samples. The extra delay between sampling and sample submission that may occur during a shoreline survey needs to be considered with respect to the location of the laboratory (ies).

If the appropriate laboratories are not available, alternative laboratory provision may be sought (e.g. analysis for stable determinands may be undertaken by a laboratory located elsewhere, even in another country) or the appropriate laboratory capability will need to be developed.

Explanation

It is necessary to ensure that appropriate laboratory capabilities have been identified prior to undertaking a growing area assessment and developing sampling plans for the relevant indicators and/or hazards. Where gaps are identified in such capabilities, appropriate action can be taken to develop such capabilities, identify appropriate alternative laboratories, or determine an alternative approach to monitoring (e.g. basing assessment of *V. parahaemolyticus/V. vulnificus* risk solely on the basis of air/ sea temperatures and salinity without including a bacteriological testing component).

2.11 COST BENEFIT ANALYSIS

Recommendation

An estimate of the overall medium-term cost for the programme for a growing area should be determined. This should include the growing area assessment, primary monitoring, initial review and the first three years of ongoing monitoring. All relevant costs should be included: central responsible authority; local authority (where relevant); industry contributions to the programme; sampling; sample transport; laboratory analyses; and surveillance. Where there are other direct costs, such as those associated with the application of animal health or environmental regulations, these may also be included. The costs should include the development of any capabilit(y/ies) or capacit(y/ies) necessary for the programme to be implemented.

An estimate of the benefits over the same period should also be determined. This should include the value at first sale together with any other benefit to the immediate community, such as the benefit of additional local employment and any additional value provided by local processing or wholesale facilities. If the sanitation programme is partly or wholly intended to provide access to export markets, the additional value provided by that access should be taken into account.

A significant aspect of a bivalve mollusc sanitation programme is the public health protection that it provides. For recreational gatherers, this is the only benefit. The protection may not just apply to immediate consumers of a product but also to others in the community who may be exposed to infection from cross-contamination to other foods or consumers who become infected – this secondary protection applies to pathogens only, and not biotoxins, chemical contaminants or radionuclides. The risk of cross-contamination or person-to-person infection varies between pathogens.

An estimate should be made of the financial benefit to the community of the public health protection aspects of the programme, An appropriate inflation factor should be applied to each estimate over the period covered by the estimates.

The outcome of the analysis should be an evaluation as to whether the cost of a growing area sanitation programme is likely to exceed, be the same as, or be less than the identified benefits of the programme.

Other considerations

Detailed data may not be available for some or all items necessary for a cost-benefit analysis, especially for areas that are not yet in production. Wherever possible, determine best estimates in order to enable a quantitative analysis to be undertaken, and identify any assumptions that have been made. Where it is not possible to even make estimates, it may be necessary to perform a semi-quantitative analysis or descriptive assessment.

Published estimates are available in the scientific literature of the financial cost to society of gastrointestinal illness. These estimates tend to have been determined for countries in northern Europe and North America and may not be applicable to other parts of the world. The application of these costs to the protective benefit of a bivalve growing area sanitation programme will need estimates to be made of both the number of illnesses that may arise in the absence of any controls and the proportion of illnesses that will be prevented by the application of the programme.

Explanation

The estimates will, at least, allow transparency of the likely cost of the programme versus the value to be gained its application. Where appropriate according to local regulations, this will allow the responsible authority to determine whether further work on a programme for the growing area constitutes a justifiable expenditure of public resource. Where a significant portion of the cost of the programme is to be borne directly by the industry, the estimates will allow the intended harvester(s) to decide whether to proceed.

2.12 CONCLUSIONS AND RECOMMENDATIONS

Recommendation

The outcome of the risk profile should be a summary of the key points relating to the growing area together with the key conclusions and recommendations that result from those key points. Any specific gaps in knowledge (e.g. local epidemiological data) should be identified at this stage and whether any particular initiatives should be undertaken to fill these gaps.

The first decision to be made is whether to proceed any further with the sanitation programme for the growing area. There are two main reasons why a decision may be made to stop the process at the growing area risk profile stage:

> The gaps in knowledge are so great that no valid conclusions and recommendations can be made. In this case, the growing area risk profile will need to be updated and completed once the gaps have been satisfactorily addressed.

> Any significant food safety risks have been identified such that expending further resource towards classification and monitoring of the growing area cannot be justified. For example:

  > The level of faecal pollution is likely to be so great that the area is unlikely to achieve a classification status that will allow harvest.

  > The presence or level of a pathogen is likely to be unacceptable and the intended or available post-harvest treatment processes will not reduce the risk to acceptable levels.

  > Levels of one or more biotoxins, chemical contaminants or radionuclides are likely to be above acceptable limits all or most of the time or for so great a proportion of the year that no beneficial harvest can be undertaken.

> The cost: benefit analysis has concluded that the cost of the programme cannot be justified in terms of the benefits that are likely to be realized. However, it may be that regulations or central government or local authority policy requires that a programme be put in place even if a cost-benefit analysis is unfavourable.

If a decision is made to proceed to the following stages (Growing Area Assessment onward), the boundaries of the assessment area should be defined to take into account the catchments in the vicinity of the intended harvest area[15] together with the area of the marine environment over which it is estimated that transport of contaminants may occur to the vicinity of the bivalve mollusc resource(s). There is a need to err on the side of caution with respect to preliminary estimates of contaminant transport distance that may be available in order that the assessment area includes all relevant potential sources of contamination.

The conclusions and recommendations should include a list of any hazards specific to the growing area that will, or may, need to be addressed in any further assessment and monitoring procedures. The list of hazards will be that determined according to the recommendations given in Section 2.9.

Other considerations

It should also be identified at this stage if there are any needs for developing capability relating to the programme: e.g. the need for regulations and competent authority ability to implement an effective programme, a need for training for the local food safety personnel or the local laboratory facilities, especially if these have not been involved previously in a bivalve mollusc sanitation programme. If the area is to be subsequently classified and monitored, appropriate guidance and/or

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[15]   The area identified by the bivalve industry for harvest of the bivalve resource. This is usually smaller than the assessment area and may be smaller than the designated growing area.

training on bivalve mollusc sanitation should be provided to commercial and/or recreation harvesters in order to enhance their contribution to the safe harvest of bivalve molluscs.

There is also a need to consider whether the originally identified assessment area is still appropriate or whether it needs to be amended prior to initiating the growing area assessment. The most relevant items of information for this purpose are:

> The location and extent of the aquaculture and/or wild harvest areas – based on both the location of the bivalves and the area(s) of intended harvest.

> The catchment area(s) of watercourses potentially impacting on the growing area.

> The estimated pollutant transport distance in the marine/estuarine environment.

It is important that the extent of the assessment area is large enough to encompass all potentially relevant contamination sources and environmental influences relevant to the ensuing assessment and associated recommendations. The growing area that is subsequently defined is very likely to be much more limited than the assessment area.

However, defining an assessment area that is too large may result in a considerable increase in the amount of information and data that has to be acquired and assessed and will also increase the complexity of the assessment.

Explanation

The conclusions and recommendations of the risk profile are the key part of the risk profile in that they determine whether subsequent parts of the sanitation programme are to be undertaken for an area and, if so, the basis for taking those parts forward.

2.13 DOCUMENTATION OF GROWING AREA RISK PROFILE

Recommendation

The supporting information together with the conclusions and recommendations from the Growing Area Risk Profile should be explicitly documented. There should be traceability through to the conclusions and recommendations from the supporting information.

Other considerations

The documented Growing Area Risk Profile should be available to relevant staff of the responsible authority and to stakeholders.

Explanation

It is important for representatives of the responsible authority and stakeholders to be aware of the outcomes of the Growing Area Risk Profile and the information on which these were based. The documentation also provides the basis for subsequent reviews.

CHAPTER 3
GROWING AREA ASSESSMENT

The growing area assessment takes into account information and data additional to that obtained for the risk profile, including practical observations recorded during a shoreline survey. This relates to the assessment area identified as one of the outcomes from the Growing Area Risk Profile.

The components of the Growing Area Assessment are:

> additional data gathering;

> shoreline survey;

> indicator/hazard survey;

> data analysis and assessment; and

> outcomes, namely:

> > extent of the classified growing area;

> > recommendations for primary monitoring;

> > risk management planning; and

> > documentation.

The relationship between these components is shown in Figure 3.1.

The recommended approach given in this section assumes that, either due to existing regulations or due to the outcome of the Growing Area Risk Profile, it is necessary to assess the growing area in relation to a wide range of hazards, including those relating to enteric pathogens. If the outcome of the risk profile identifies a limited range of potential hazards, or if a policy decision has been taken to concentrate on one or a small number of hazards of primary health significance, it may be appropriate to restrict the scope of the growing area assessment. For example, if the outcome of the risk profile, or a policy decision, determined that it was only necessary to address *V. vulnificus* in bivalves intended to be eaten raw, the elements of the assessment relating to sources of faecal and chemical contamination would not be relevant. Again, if biotoxins, or a single biotoxin group (such as PSP), were

determined to be the only hazards of importance, then sources of faecal and chemical contamination would not be relevant to the assessment process. If enteric viruses were the sole identified hazard of concern, the Growing Area Assessment would concentrate on sources relating to human faeces (N.B. hepatitis E virus may be related to animal sources). Lastly, if one or more chemical contaminants were identified as the only hazard(s), sources of faecal contamination would not be of interest unless some of these might contain relevant industrial effluent. Figure 3.2 shows the principal contamination sources and environmental factors that need to be considered for each major group of hazards during the Growing Area Assessment.

It must be emphasized that the relationship between hazards, and potential sources or environmental conditions is iterative. The range of relevant hazards identified during the Growing Area Risk Profile will dictate the range of potential sources that may be of relevance to the assessment and subsequent monitoring and management. However, sources or environmental conditions found during the assessment process may identify that hazards that were not determined during the Growing Area Risk Profile should be taken into account in the sanitation programme for the growing area. For example, an industrial effluent may be identified that may discharge significant amounts of one or more chemical contaminants that was (were) not previously identified as hazards.

The Growing Area Assessment will usually involve the acquisition of much more detailed data and information than was obtained in support of the Risk Profile. The targeting of this additional data gathering exercise will be informed by the summary information gathered in support of the Risk Profile. However, care needs to be taken: just because information relating to specific hazards or sources of contamination were not identified in restricted information gathering exercise undertaken for the Growing Area Risk Profile does not necessarily mean that such hazards or sources were not relevant to the assessment and growing area. . Figure 3.2 shows the main sources and factors relevant to the major hazard groups. Some of the sources and factors identified for chemical contaminants will also be relevant to radionuclides.

The information and data gathered for the assessment may identify hazards that were not considered in the risk profile. If so, the risk profile should be amended accordingly. The assessment and management process is iterative and modifications to individual elements need to be made as necessary. This is formalized in the review procedure but should not be confined to that process.

A template for a Growing Area Assessment is given in Annex 2.

FIGURE 3.1 **COMPONENTS OF THE GROWING AREA ASSESSMENT**

ADDITIONAL DATA COLLECTION

SHORELINE SURVEY

INDICATOR/HAZARD SURVEY

DATA ANALYSIS

ASSESSMENT

OUTCOMES

DOCUMENTATION

FIGURE 3.2 **PRINCIPAL SOURCE AND FACTOR CONSIDERATIONS FOR THE MAJOR HAZARD GROUPS**

HUMAN ENTERIC PATHOGENS	ANIMAL ENTERIC PATHOGENS	MARINE VIBRIOS	BIOTOXINS	CHEMICAL CONTAMINANTS
Continous sewage discharges	Farm animal polpulations	Concentration of potentially pathogennic species	Occurrence of potentially toxic phytoplankton	Industrial effluents
Intermittent sewage discharges	Animal waste storage locations	Proportion of pathogenic strains	Occurrence of biotoxins in bivalves	Landfill sites
Private sewage discharges	Slurry spreading locations	Seawater temperature	Seawater temperature	Mining
Direct defecation to land	Terrestrial wild animal populations	Seawater salinity	Seawater salinity	Shipping & boating
Sewage sludge spreading	Marine wild animal populations			Marine waste disposal sites
Rainfall-dependent discharges (or run-off)	Wild bird populations			Geological sources
Shipping & boating	Rainfall mediated run-off			Contaminated sediments

Hydrography

3.1 ADDITIONAL DATA GATHERING EXERCISE

The amount of data gathered for, or assessed in, the Growing Area Risk Profile is limited to that necessary for that initial process. The data gathering exercise undertaken for the Growing Area Assessment should be much more detailed so as to allow a full assessment on which to base the subsequent monitoring and management. In general, it should concentrate on potential sources of contamination relating to the hazards identified in the Risk Profile (where such hazards are associated with contamination sources) and on factors that affect the occurrence or impact of the hazards. Usually, the process will consist of obtaining information and/or data on the potential sources and environmental factors for the defined assessment area. Depending on local circumstances, and the level of detail obtained for the risk profile, little or no additional information and data may be needed on some elements of the Growing Area Assessment. For example, in the case of assessment areas remote from centres of human population, sufficiently detailed information on all of the potential sources of human pollution may have already obtained in support of the Growing Area Risk Profile. For other aspects, such as potential inputs from animal sources, it may be identified that the summary information obtained for the Growing Area Risk Profile is all that is available (other than any site-specific records made during the shoreline survey). Determining what additional relevant information and data may be available is the first necessary step for each element of the additional data gathering exercise.

Where bivalves are grown in artificial ponds or land-based tanks fed with seawater, the location of the seawater feed to the pond(s) or tanks(s) can be considered as the source of contamination. However, it is necessary to consider the contamination sources that may potentially impact on the seawater intake point as these will affect the contaminant content and variability of the seawater feed. The seawater intake for some types of system may be able to be shut off during contamination events and this may be taken into consideration for the assessment, monitoring and management of such systems if shut off can be reliably undertaken before any contamination enters the system.

3.1.1 SOURCES OF CONTAMINATION

3.1.1.1 Untreated community sewage discharges

In some countries, community sewage collection (sewerage) systems (see Section 3.1.1.3) or other wastes may discharge direct to the aquatic (watercourse or marine) environment without any prior treatment.

Recommendation

Obtain the following information:

> permit (consent)[16] no. (where relevant) and, if different, the operating organization's identifier;

> discharge name (where relevant);

> discharge location (geographical co-ordinates and whether to watercourse, marine environment, land;

> whether the discharge is pumped or operates under gravity;

> flow records (average, maximum and minimum); and

> permitted or actual levels of relevant hazards (where relevant).

Other considerations

Where no information is available on permitted or actual flows, the maximum may be estimated from the volume of the pipe or, for pumped discharges, the number of pumps and their rated flows.

Where no information is available on microbiological quality, estimates may be made on the basis of typical values from the scientific literature (e.g. Kay, *et al.*, 2008). Estimates of the concentration of other hazards in the discharge may be made on the basis of known loadings to the collection system and the total estimated volume rate of discharge from the outfall.

In some countries, consideration will need to be given to informal untreated community discharges, e.g. those associated with informal settlements (e.g. shanty towns). Information on the presence of these may be obtained from local sources and during the shoreline survey.

Explanation

In some countries, regions or areas, sewage may not be subject to any form of treatment prior to discharge into the environment. Such discharges pose a greater risk of contamination to the bivalve resource(s) as there will be no reduction in the concentration of relevant hazards due to treatment processes. There is less likely to be relevant information on flows and hazard loadings for such discharges than of those from treatment works (see Section 3.1.1.2). An assessment of likely impact usually has to be based on estimates of volume rate of discharge and hazard concentration.

Paradoxically, untreated sewage discharges do have the advantage that there is no differential inactivation of faecal indicator bacteria and enteric pathogens in a treatment process, only in the environment after discharge. Faecal indicator bacterial concentrations measured at the bivalve resource(s) may therefore be more directly indicative of pathogen risk than when the resource(s) is(are) affected by treated sewage.

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[16] A number associated with the treatment works or discharge given when authorisation is given by the environmental regulator – this authorization may be termed a permit or a consent.

3.1.1.2 Sewage treatment works

Sewage treatment works may also be referred to as wastewater treatment[17] works. However, the latter term also covers treatment works for farm, food processing, industrial and mining wastes. Discharges from these other treatment works may contain one or more hazard groups such as enteric pathogens and chemical contaminants. Which hazards are present will depend on the content of the influent material and the effectiveness of any treatment. These other treatment works and the associated discharges are considered in later subsections.

An overview of wastewater (primarily sewage) collection and treatment is given at: http://www.unep.or.jp/ietc/Publications/Freshwater/SB_summary/

Recommendation

Obtain the following information:

> permit (Consent) no. (where relevant) and, if different, the operating organization's identifier;

> treatment works name (where relevant);

> the location of the treatment works;

> types of influent to the works (domestic, industrial, storm water, septic disposal, etc.);

> population equivalent[18] served;

> level of treatment (raw, primary, secondary, and including whether disinfection[19] is included);

> the occurrence of any split flows or bypasses (i.e. where all or some of the flow can receive a lower level of treatment);

> flow (average and variability), microbial content (average and variability);

> if the microbial content is not available, information on sanitary characteristics (Biochemical Oxygen Demand (BOD), Ammonia and Suspended Solids) will give a general indication of effluent quality;

> known malfunctions;

> the locations of associated discharges (geographical co-ordinates and whether to watercourse, marine environment, land (including soakaway) wetland to lagoon); and

> whether the discharge is limited to certain times (e.g., tidally phased).

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[17] The process of removing or reducing contaminants from wastewater, typically through physical, chemical, and biological processes (to produce treated effluent).

[18] One population equivalent (p.e.) means the organic biodegradable load having a five-day biochemical oxygen demand (BOD5) of 60 g of oxygen per day.

[19] Disinfection is a process that destroys, inactivates, or reduces microorganisms in wastewater by means other than simple settlement or biological activity. This may be applied as the last stage of treatment in the wastewater treatment processes.

Other considerations

The amount of additional information that is gathered will depend on the potential significance of the discharge to the assessment area (e.g. with respect to microbiological quality or the impact with respect to other hazards). An initial assessment of the potential degree of impact may be made based on the information listed under the Recommendation section. Priority for the acquisition of additional information will include known implication of the works in pollution incidents within the assessment area or in bivalve-or recreational water activity-associated outbreaks.

Such additional information may include:

> process design and operation information:

  > additional useful process information includes design flow and hydraulic capacity of the works and expected range of flow under dry weather and wet weather conditions, such as the average daily flow and peak hourly flow. The disinfection type, usage, operation and maintenance, and backup systems and any additional measures such as influent storage capacity should also be described;

  > whether storms/high flows can disrupt the level of treatment;

  > for treatment works including disinfection, additional information may include usage under average and peak flows, including exposure/contact time, intensity/energy output (for UV systems), or chlorine residual (for chlorine systems), membrane size and frequency of backwash cycle (for membrane systems).

> level of monitoring by the treatment works (staffing and through automated monitoring and alarm systems);

> compliance with permit (consent) requirements (e.g. flows, specified limits for effluent quality, limitations on the operation of bypasses, disinfection dosage. Data for the previous three years).

A procedure to assist the assessment of a sewage treatment works is given in Annex 3.

The microbiological quality of discharges from sewage treatment works may not be available. If not, estimates may be made on the basis of typical values from the scientific literature (e.g. Kay *et al.*, 2008).

For treatment works where there are significant concerns of the impact of a hazard on the bivalve resource(s), the responsible authority may consider assessing the efficiency of the treatment works for that hazard. For microbiological impacts, this may be determined using a combination of faecal indicator bacteria and either specific pathogens of concern, or MSC. The determination of efficiency using MSC is described in Annex 13.

Treated sewage effluent may be used for fertilizing or water land areas, the intent depending on the level of treatment and thus the nutrient content. This may result in

a risk of run-off of faecal indicators, pathogens, or chemical contaminants following rainfall. In some countries treated effluent may only be used for such purposes if it meets certain limits.

Explanation

If community sewerage systems are present in an area, discharges associated with sewage treatment works will often be the principal continuous sources of human faecal contamination of the aquatic environment, potentially containing bacterial and viral enteric pathogens. These may, directly or indirectly, impact on the water quality in the growing area. The characteristics of the influent to the works, the volume of discharge and the treatment level within the works will affect the quality of the effluent under normal operating conditions. Normal operating conditions means that the treatment works is operating fully within the design specifications, including design flows; treatment stages; disinfection; as well as compliance with permit conditions that relate to the treatments works effectiveness in reducing pollutants including enteric pathogens. It is also necessary to consider the variability of the volume and quality of the effluent, including the potential for operating outside of design specifications and other types of failure events that could occur, as the assessment of potential impact should normally be targeted towards the worst case situation. If the worst case occurs very infrequently, the associated risk may be managed by means of an event management plan and the general assessment may be based on the expected operating conditions. Examples of the types of event that may occur and should be considered within an event management plan are given in Annexes 13 and 14.

3.1.1.3 Sewage collection (Sewerage) systems

Recommendation

Obtain data on the location of pumping stations (and any associated emergency overflows), storm water storage tanks (and associated outfalls) and combined sewer overflows (CSOs). Also the location of such intermittent overflows associated with sewage treatment works.

Other considerations

Where possible obtain information on permitted operation of any intermittent discharges, such as the conditions under which they are allowed to operate. For emergency overflows, obtain information on the likely maximum flow and discharge notification procedures. For storm associated discharges (including storm tanks and CSOs), obtain information on the predicted, or preferably, actual frequency of spills together with spill volumes (where these are recorded)

Where spill occurrences and/or volumes are not recorded, the potential impact of intermittent discharges may be assessed using estimations of the flow and spill frequency. Worst case flow estimates may be based on the dimensions of the sewer

or outfall or, for pumped overflows, the number of pumps and their rated flows. For overflows from sewers that are physically above the base of the sewer, the default pass-forward flow (below the level of the overflow) is subtracted from the estimated total flow to obtain the worst-cased spill flow.

It is also necessary to determine whether any industrial facilities discharge trade waste to the sewerage systems and thus whether any chemical hazards may be associated with either the continuous and/or intermittent sewage discharges.

Explanation

In areas served by community sewerage systems, and where there is treatment of continuous sewage discharges, the operation of intermittent discharges will lead to significant additional human faecal contamination of the aquatic environment and these may, directly or indirectly, impact on the water quality in the growing area. It is therefore important that the potential operation of such discharges are taken into account in determining the likely faecal impact on the growing area. Industrial discharges entering community sewerage systems may contribute chemical contaminants to the assessment area via either continuous or intermittent discharges.

Separated sewage collection systems, where surface water is kept separate from sewage, may have flow rates that do not vary markedly with rainfall, snowmelt or ice melt events. The sewerage system may then not have intermittent discharges to cope with the non-septic flows but may still have emergency discharges that may operate due to pump breakdown. Surface water discharges may themselves be sources of contamination due to land run-off and intentional or accidental cross-connections from the sewage collection system.

The microbiological quality of discharges from intermittent discharges may well not be available. Estimates may be made on the basis of the knowledge of the quality of the influent to a treatment works downstream of the intermittent discharge (where relevant) or based on typical values from the scientific literature (e.g. Kay, *et al.*, 2008).

3.1.1.4 **Private sewage treatment works**

Recommendation

Obtain data on the following:

> private sewage treatment works, type of works, level of treatment, volume treated per day, location of discharge, maintenance requirements and any record of malfunctioning; and

> location of any associated overflows, together with any controls on operation of these, or records of occurrence.

Explanation

In areas where there is no existing community sewerage system, where there are capacity limitations on the community sewage collection system and/or sewage

treatment works, or where connection to an existing system is not practical, planning controls or environmental regulations may require the provision of a private sewage treatment works for a building development. Such a development is often a group of houses but may be a larger housing/apartment or industrial development. In such cases, the treatment is often provided by the installation of a package sewage treatment works (also termed package plants), which contain several stages of a treatment process in a prefabricated unit.

3.1.1.5 Other private sewage treatment/handling facilities

Recommendation

Obtain data on the following:

> community or private septic tank locations, type of septic tank, type, volume treated per day, location of discharge (including whether to land, watercourse or marine environment) and any record of malfunctioning;

> location of cesspits together with information on the frequency of emptying and destination of the emptied contents. Any records of malfunctioning;

> location and use of drop pit latrines for human defaecation;

> soil/drainage suitability for subsurface discharge (for soakaway drainfields);

> where records can be obtained, it may be useful to compare water use records with design of volume of water, assuming volume treated per day; and

> seasonality of use; and

> information on the nature and frequency of maintenance of septic tanks, emptying frequency of cesspits and drop pit latrines (or their replacement with new drop pits).

Other considerations

In areas where septic systems are potentially a significant source of pollution additional information may be useful in a more detailed assessment of septic system impact. This information (if publicly available) includes, age of septic system, soil classification, soil percolation rate used in the design, capacity of septic system, and water usage records.

Explanation

Septic tanks may discharge to the aquatic environment (watercourses, estuary or coastal environment) or go to soakaway (including drainage fields). Community septic tanks (including community Imhoff tanks) will generally have a greater potential impact on the assessment area than private septic tanks, due to both the greater amount of faecal material being treated and the greater potential for pathogens to be present (e.g. norovirus will only be excreted by a proportion of the connected population). However, even small septic tanks will potentially have an

impact in the vicinity of any discharge and will be the source of pathogens if one or more of the small population using the system are excreting the pathogen.

The treatment level achieved by the septic tank itself will vary according to the design, operation and degree of maintenance and will usually vary between the equivalent of primary and secondary treatment. The effluent may then be subject to further treatment, e.g. by discharging to a soakaway, where physical filtration, and potential biological activity, will further reduce the level of pathogens. Septic tanks discharging directly to the aquatic environment may be assessed in the same way as a sewage works output serving the same population and of the same equivalent treatment level.

The life expectancy of a septic tank discharging to soakaway can vary from a relatively short time if installed in a wet area or poorly drained soil, to a longer time if properly installed in more suitable, well drained soils. Comparing the age of the system with the soil classification may give an indication of which septic systems may be closer to the end of their useful life. It is also useful to compare the soil classification to soil percolation rate used to size the system to ensure that they are consistent. A percolation test indicating high drainage in soils that are classified as poorly drained can indicate that a system was under designed. In older more established communities with changes in population and usage an older system may not be designed to handle the current use. Overuse of the system is typically the most common factor in failed septic systems. A comparison of the capacity for which septic systems were designed with actual water usage records can help to locate systems where overuse might be a problem.

Cesspits are usually simple collection systems for sewage. They are then emptied and the collected sewage discharged elsewhere, often to the influent stage of a sewage treatment works or to a lagoon where it is aged and then discharged to land or soakaway. As with most types of septic tanks, cesspits need to be periodically de-sludged. Properly constructed, maintained and emptied cesspits should pose no risk of contamination to the assessment area. However, leaks, overflows and spills during the emptying process can result in contamination, either ongoing or sporadic.

Faeces voided in drop latrines may go to the aquatic environment, the shoreline, or to pits. Which of these, and the size of the population using the latrines, will influence the potential impact.

Explanation

Private sewage systems may predominate in some areas or may be significant additional sources of human faecal contamination in areas also served by community sewerage systems. Although the volumes of sewage may be much less than those involved in community systems, they will still be significant sources of contamination to the growing area if there are either a large number of such systems and/or if they are in close proximity to the growing area. The impact will be greater if there is a malfunction. In general, due to the lower connected population, the presence of any enteric pathogen content will be more intermittent than with community sewerage

systems. However, it is not usually possible to predict when such pathogens will be present although this will be more likely when the occurrence of the pathogen in the community is high. Septic tank systems associated with holiday homes may only be used on a seasonal basis: in such cases, the system will not operate properly for several days after use until the flow and content has stabilized. In other situations, a stabilized septic tank system may be overwhelmed by a sudden increase in the user population: e.g. a large house or other facility hosting a function attended by a large number of people. Rainfall, especially when heavy, may cause overflow or malfunction of septic tank systems, as well as overflow of cesspits and drop pit latrines if these are not protected from the entrance of rain. Rainfall may also cause failure of effluent disposal fields if these are not constructed properly.

Hay, McCoubrey and Zammit (2013) identified that the following issues with septic tank systems may have contributed to the occurrence of norovirus outbreaks related to oyster consumption:

> inadequate design;

> inappropriate installation;

> inadequate maintenance;

> unapproved modifications; or

> system failure (especially with regard to effluent disposal fields).

3.1.1.6 Direct human defaecation

Recommendation

Where relevant, determine estimates of the occurrence and location of direct human defaecation to land (or water), together with known locations and timing of nightsoil spreading.

Where direct data is not available, the number of people using private septic systems, cesspits, drop pits and/or direct defaecation may be estimated by subtracting the population known to be connected to the community sewerage system from the total population of the area. The type of non-community disposal is then inferred from knowledge of local habits.

Explanation

It is estimated that direct human defaecation to land is still practiced by 1 billion people in the world (WHO and UNICEF, 2014). Nightsoil spreading on arable and horticulture areas is also practiced in some countries. Both activities will contribute faecal indicator bacteria and human enteric pathogens to the environment and may have an impact on marine and estuarine waters, directly or indirectly, after rainfall. The occurrence of, and risk from, pathogens will depend on both the prevalence in the human population and the prevalence of direct human defaecation and/or nightsoil spreading.

3.1.1.7 Sewage sludge application

Recommendation

In areas served by community sewage treatment works, or those where facilities treat septic tank sludge or cesspit contents, obtain information on the location of the application of sewage sludge to land, periods when this takes place, any treatment applied to the sludge prior to application and any constraints that may be applied by regulators.

Explanation

Sludge obtained from sewage treatment is often applied to land as a fertilizer. Such sludge may contain a range of pathogens and also chemical contaminants. Sludge may be subjected to treatment such as long-term storage and/or pasteurization prior to application in order to reduce the level of some pathogens. Constraints may be applied on the application of sludge to land in order to reduce environmental impact. Potential constraints include restriction of application to certain times during the year and prohibition of application prior to forecast wet weather.

3.1.1.8 Shipping and boating activity

Recommendation

Determine estimates of the occurrence or shipping and boating activity, including the occurrence and usage of ports/harbours, marinas and concentrations of moorings or anchorages. Estimate the approximate likely occupancy (numbers of people on board) of vessels by day and overnight. Determine any seasonality associated with these activities.

Other considerations

Identify whether any restrictions on discharge of human waste apply to vessels, including formal no-discharge zones, and determine any information on compliance with those restrictions. Identify whether there are any commercial or recreational fishing activities that may result in discharge of faecal waste from boats. Also consider whether any discharges may occur from floating structures associated with bivalve or other aquaculture or wild harvest activities.

There are recognized standards for marine sanitation devices for larger vessels (e.g. see http://www.uscg.mil/hq/cg5/cg5213/msd.asp). As with sewage treatment works, the actual performance may vary with type, age and level of maintenance. Information on these aspects is important to determine if larger vessels are moored, with crew onboard, for any length of time within the assessment area. Depending on local regulations, smaller vessels may have the following waste facilities:

> none (with possible overboard disposal of faecal waste);

> heads (onboard toilets) with:

> direct discharge;
> maceration systems;
> chemical toilets;
> composting toilets;
> marine sanitation devices (smaller versions of those fitted to larger vessels); or
> holding tanks.

Determine whether there are any local regulations requiring treatment systems or holding tanks for waste being fitted in smaller vessels. Where holding tanks are fitted, it is necessary to determine whether they could be discharged within the assessment area. This will partly depend on the provision of any holding tank pump out facilities, the functioning of these, and whether a large proportion of boats use such facilities.

The discharge of ballast water from larger vessels may introduce potentially toxic algal species, marine vibrios and chemical contaminants into the receiving environment. While the discharge of ballast water comes under an international convention, adoption of this has been limited and thus, unless local regulations are in place, and enforced, such events should be considered if larger vessels use the assessment area.

Chemical contaminants may also be introduced by fuel spillages and by the use of antifouling paints. The potential level of contamination from both sources also depends on the presence of local control regulations and the effectiveness of enforcement.

Some types of fishing activity (e.g. dredging) and bait gathering may cause re-suspension of marine sediment containing pathogens and/or chemical contaminants.

Explanation

Discharge of faecal material from ships and boats may directly or indirectly impact on the growing area. Discharges from large ships come under the auspices of international pollution control agreements. However, discharges of either treated or untreated faecal material may take place from smaller ships and boats. In addition, discharge of faecal material may take place from toilet facilities associated with floating structures. The existence of international or local regulations do not necessarily mean that these are followed and information on local adherence and practices should be taken into account when making an assessment of the likely impact within the assessment area.

3.1.1.9 Land use and agricultural activity

Recommendation

Obtain information on the following:

> mining activities, including locations of solid and liquid waste disposal;
> location of major land-use types;

> livestock farm locations (including smallholdings) and usage;

> feedlot locations and usage;

> known concentrations of extensive grazing of farm animals and seasonality;

> location of abattoirs and any associated effluent and slurry storage and disposal;

> locations, volumes and construction details of slurry (animal faecal waste) storage systems together with any requirements for spill-avoidance procedures and any known spill events;

> known locations and timing of slurry spreading. details of any treatment procedures (e.g. long-term storage) prior to application or restrictions on application of slurry to land;

> known locations and timing of artificial fertilizer, herbicide and pesticide application; and

> known locations and timing of external animal treatments, including those used in finfish aquaculture.

Explanation

Mining activities may contribute high levels of chemical contaminants to the environment and these may enter the coastal or estuarine environment, either directly or indirectly (e.g. via watercourses). The chemicals of concern may not be those that are the target of the mining but may be others related to the local geology. Management measures may be in place to reduce environmental impact, e.g. treatment of waste process water. Such measures need to be identified as they may be relevant to assessment of the likely impact of the hazard and may require an expected event management plan in case of failure of the management measure(s).

Herbicides, pesticides and animal treatments contribute chemical contaminants to the environment. They will differ markedly in potential toxicity and solubility. Minor components may be as, or more important with respect to the bivalve assessment than the major component(s).

Fertilizers, whether artificial or natural, may also contribute chemical contaminants to the environment. Minor components of a fertilizer may be of greater concern than the main component(s). For example, superphosphate fertilizers have been known to contain significant levels of cadmium.

Historical mining and chemical usage needs to be considered as some contaminants may persist for long periods in the environment.

Animal faeces are a source of faecal indicator bacteria and a range of pathogens. Large concentrations of animals, or collections or animal faeces (e.g. as in slurry storage) poses a much greater risk than extensive grazing of small numbers of animals. Depending on the situation, the location may be considered as a point or diffuse source of pollution.

Abattoirs pose two separate forms of potential pollution. The first relates to the gathering together of large numbers of living animals and the faeces produced by those animals. In this respect, they act like other concentrations of farm animals. The second relates to the waste from the slaughter and any cutting processes. This waste will also contain faecal indicator bacteria and pathogens.

Land-use types may indicate the potential for faecal contamination. Fertilizer, potentially including sewage sludge or animal slurry, is likely to be applied to improved pastureland and arable land. Increased run-off, including high concentrations of faecal indicator bacteria, may occur in forestry areas after significant felling activity.

3.1.1.10 Other human activities

Recommendation

Information should be obtained on disposal of industrial waste, including liquid discharges from factories, and solid waste disposal, as well as domestic refuse disposal sites. This should include the location of discharge/disposal, volume/amount involved and the actual concentration (or permitted levels) of contaminants of relevance to the hazards identified in the Risk Profile.

Other considerations

Liquid and solid wastes associated with industrial and other activities may contain chemical contaminants that may be accumulated in bivalve molluscs if they enter the marine or estuarine environment. Effluent from food factories and timber mills may contain high concentrations of faecal indicator bacteria. That from food factories may also contain pathogens: in the case of seafood processing plants, the waste may contain pathogenic vibrios as well as other bacterial pathogens. The effluent from some food factories may be subjected to some form of treatment, usually to reduce the environmental impact from the organic content.

Domestic refuse disposal sites may also pose a source of chemical contaminants, faecal indicator bacteria and potentially pathogens if there is liquid seepage from the site. Landfill sites may contain disposed contaminated toilet paper, soiled nappies (diapers) and dog faeces. In addition, they may pose an indirect source of faecal indicator bacteria and pathogens as they often attract large numbers of seagulls (this may also apply to open sewage treatment works and areas of sewage sludge and animal slurry application).

Information should also be sought on any sources of radioactive discharges (e.g. associated with nuclear reactors) relevant to the growing area. The potential impact area of such discharges may be great, and the half-life of some radionuclides can be very long and these aspects need to be considered in the assessment process. Information on sources should be supplemented, where possible, with data from environmental monitoring.

Salt mine and salt pan discharges may cause significantly increased salinity in the vicinity of the discharge point. Other types of wastewater discharges may result in significantly depressed salinity levels and/or increased temperatures in the vicinity.

Dredging of marine sediments may cause significant re-suspension of particulate material containing chemical contaminants, radionuclides, cysts of potentially toxic phytoplankton and/or pathogens. The significance depends on the presence and concentration of the hazards in the sediments, the degree of re-suspension caused by the specific dredging process in use, and the degree of dispersion of the re-suspended material in relation to the bivalve resource.

Marine dumping of waste has been phased out in many parts of the world but this needs to be determined for the area in question. Dumping of marine sediments dredged elsewhere (e.g. from ports and shipping channels) is often allowed and these may contain chemical contaminants, radionuclides, cysts of potentially toxic phytoplankton and/or pathogens, depending on the source material.

Explanation

A wide range of human activities may contribute significant levels of faecal, chemical and/or radiological contamination to the assessment area and these may potentially impact on the growing area. Such sources may add to the contamination arising from other identified sources. Changes in salinity and/or temperature may be relevant to the growth of potentially toxic phytoplankton and marine vibrios.

3.1.1.11 **Wild animals and birds**

Recommendation

Identify concentrations of wild animal populations, including marine mammals and birds including their main areas of use and any associated seasonality.

Other considerations

Populations may be increased around feeding sites or breeding sites. Feeding sites may be natural (e.g. intertidal areas for many wading birds) or associated with human activity (e.g. concentrations of birds around landfill and other refuse disposal sites and open sewage treatment works).

Explanation

Wild animals contribute faecal material to the environment and therefore pose a source of faecal indicator bacteria and pathogens. Impact from small numbers of animals and birds will be sporadic and difficult to assess but large numbers will contribute significant amounts of faeces and this source needs to be taken into account in the growing area assessment. Birds scavenging on human wastes may pick up pathogens from those sources in addition to carrying those that are naturally found in the intestinal tract of birds (see Section 2.6).

3.1.1.12 Watercourses

Recommendation

Identify the location of all watercourses[20] within the assessment area.

Other considerations

The significance of a watercourse will depend on its loading of any particular hazard or indicator, its distance from the bivalve resource and the hydrography of the area. Some preliminary judgment on the potential impact will be necessary in areas containing a large number of watercourses in order to prioritize those for further investigation. This will normally be done on the basis of the volume rate of discharge (volume discharged per unit time), known occurrence of sewage discharges and farms, and distance from the bivalve resource.

Explanation

Watercourses act as conduits to conduct contaminants, including faecal animal and human contamination, from their source within a catchment to the marine or estuarine area and thus act as sources of contamination to the growing area. The importance of a watercourse from this perspective will relate to its loading which is a function of both the concentration of each contaminant and the volume rate of discharge of the watercourse. The concentration of each contaminant will vary according to the factors affecting the sources, the pathways from those sources to the watercourse, and whether significant sedimentation or mobilization takes place. The rate of discharge will principally vary according to rainfall and snow or ice melt (where relevant). Thus loadings will vary from year to year, season to season, and even between days (and potentially shorter timescales). Estimates should be made of the loading of each relevant contaminant identified as a hazard during the risk profile (not all hazards may necessarily be directly related to watercourse inputs). This necessitates determination or estimation of the concentration and the flow of the watercourse. Where the loading is of possible significance to the overall growing area assessment, likely variability in the loading should be determined.

Watercourses can have a number of other effects relevant to the growing area assessment:

> the discharge may continue into the marine or estuarine environment, transporting contaminants away from the mouth of the watercourse. That discharge will also modify local currents and thus the transport of contaminants arising from sources other than the watercourse;

> there will be local reductions in salinity. The degree of that reduction, and the area over which the effect is seen, will depend on the watercourse volume rate of discharge, and the depth and salinity of the marine or estuarine area;

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[20] For examples of watercourses, see footnote in Section 2.3.

> there may be modifications to temperature within the marine or estuarine area. The extent of the modification will depend on the volume rate of discharge, the depth of the marine or estuarine area and the initial temperature difference between that area and the watercourse; and

> high volume rate of discharge can shorten the contaminant transport time.

3.1.2 GEOGRAPHICAL, HYDROGRAPHIC, METEOROLOGICAL AND OTHER ENVIRONMENTAL FACTORS

Recommendation

Information and data relevant to the factors covered in Sections 3.1.2.1 to 3.1.2.5 should be obtained and recorded.

Explanation

There are a number of ways that environmental factors, in the broad sense, affect the occurrence and/or concentration of contaminants in bivalve molluscs, namely by:

> increasing the concentration of the contaminant in the source (less effective sewage treatment at high flows; higher concentration of vibrios at high temperatures or low salinity);

> increasing the impact of the source on the environment (e.g. spill from rainfall-dependent overflows; increased run-off from land);

> changing the pathway between source and bivalves (e.g. diversion of flows, change in current patterns);

> changing the survival of the contaminant in the environment; or

> changing the uptake and/or depuration rate of the contaminant by the bivalves.

Environmental factors may affect the presence and/or concentration of contaminants differently between different species of bivalve molluscs due to: biological differences between species and variation in location within the water column or sediment. In addition, some factors may be relevant to one species and not another due to differences in local harvesting season. Therefore, the effect of environmental factors needs to be considered within the assessment for each bivalve species being addressed.

The effect of environmental factors may also likely to differ between contaminants, including between faecal indicator bacteria and bacterial and viral pathogens.

3.1.2.1 Geology

Recommendation

Identify the principal rocks present in the area, primarily from the viewpoints of chemical composition and porosity, together with the soil types that are present.

Other considerations

The type and depth of soil, together with the degree of fracturing (of both the soil itself and underlying rocks) is important in determining the suitability of an area for sewage and septic tank effluent soakaways. In addition, in combination with the slope of the land and the nature of any land cover, they determine the degree of run-off that may occur.

The chemical composition of soil and/or rocks may be important in determining whether natural contamination occurs with respect to chemical contaminants such as heavy metals and arsenic. Marine sediments may also contain naturally occurring chemicals (such as heavy metals) that may be taken up by benthic bivalves or even by those growing elsewhere in the water column if the sediments are re-suspended by natural or human causes.

Explanation

The geology of the area, including soil types that are present, may determine whether some chemical hazards are naturally present in the marine or estuarine environment and may also affect the salinity and pH of the water in the growing area. Rock porosity and soil type may also affect permeability and natural filtration and so influence the amount of run-off from land and the concentration of specific contaminants, including pathogens, that may end up in the growing area, either directly or via watercourses.

3.1.2.2 Topography

Recommendation

Determine the topography of the assessment area, including the marine/estuarine coastline, land adjacent to watercourse, and the surrounding landscape.

Explanation

Topography has a complex influence on the fate of contamination in the environment and the potential for impact on the growing area. The slope of land and surface characteristics in an area will affect the amount and rate of land run-off that may occur and will have implications for the engineering of sewerage networks. It also affects the flow rate of watercourses. The shape of land affects the direction taken by watercourses and the shape of the coastline (bays, headlands and islands) will affect the direction and strength of currents in the growing area.

3.1.2.3 Hydrography

Bathymetry

Recommendation

Determine the main bathymetric characteristics of the marine/estuarine environment within the assessment area.

Explanation

The depth of an area influences the dilution of contaminants that may take place and the presence of channels influences the direction of local currents, and thus the path that water carrying contaminants may take. Therefore, the depth and shape of the area should be determined. This information is usually obtained from hydrographic charts prepared by national authorities but may be supplemented by the results of other bathymetric surveys (e.g. undertaken for port authorities, or natural resources surveys), measurements taken during shoreline surveys, and local knowledge.

Tides

Recommendation

Determine the tidal characteristics for the area, including the tide type, average tidal range at both spring and neap tides, and the variability in both spring and neap tidal range.

Other considerations

In areas with a small tidal range, tidal effects may be minimal and currents due to other factors (e.g. wind) may predominate. However, tidal currents may be significant in areas with small tidal ranges where exchange of water is limited to relatively narrow channels (e.g. between the mainland and an island, or between two islands).

Explanation

Tides affect the depth of water in an area with time, and thus dilution, as well as driving currents and sometimes causing re-suspension of sediments. In many areas, the interaction of tides with the bathymetry of an area means that the pathway taken by contaminants may vary markedly with time: at the extreme, significant concentrations of a contaminant may reach the growing area under some states of tide and none at all under other states. Tides may be diurnal, semi-diurnal or complex. Tidal ranges (difference in depth between high and low tide) may be macrotidal (>4 metres), mesotidal (2 to 4 metres) or microtidal (<2 metres). The range will typical vary between spring tides (greatest range) and neap tides (smallest range). The tidal pattern and range should be determined for the growing area. If the growing area is not located near to a tidal gauge, this may need to be determined by measurement (for example, by placing tide or pressure gauges in one or more

suitable locations in the area for a period of time (at least two weeks, ideally covering spring and neap tide).

There is an interaction between bathymetry in the growing area and the topography of the land at the coast, especially in areas with meso- or macrotidal ranges. For example, where a land bridge is present at low tide between the mainland and an island (or between two islands), the route taken by contamination from a specific source may be totally different at low tide and high tide.

Hydrodynamics

Recommendation

Determine the hydrodynamics that apply to the assessment area. This involves determining the currents that apply in the area and how these vary with tidal, meteorological (e.g. wind driven currents, which may predominate in areas with a small tidal range) and other factors (e.g. seasonal).

Other considerations

The hydrodynamics may be determined by a number of approaches. The following are given in increasing order of complexity and resource requirement. In general, the simplest approach that yields the required level of information should be used. However, information and data relating to the hydrodynamics may also be used in the determination of buffer zones (see Section 5.7). The approaches are:

i. *Use of hydrographic charts* – these often contain information on the principal tidal streams at specific locations.

ii. *Use of tidal stream software* – this will usually display the direction and speed of tidal streams at specific locations and times.

iii. *Drogue tracking* – recording the transport of a physical object or objects within the assessment area. This will usually have to be done under a range of conditions tidal and meteorological conditions.

iv. *Simple hydrodynamic modelling* – using off-the-shelf models with appropriate input of depth and boundary state tidal information. Ideally, it should also be possible to add the effect of different wind directions and strengths. A particle tracking model may be superimposed on the hydrodynamic output in order to determine the fate of one or more contaminants from one or more sources over a range of conditions.

v. *Tracer studies* – the use of dyes, bacterial spores, bacteriophages or labelled particles to follow the movement of water in an area. The material is released at a specific location and then followed as it moves through the water. For dyes, this may involve simple visual tracking of a plume or measurement of concentration at a number of locations (and possibly depths) within the assessment area. For dyes and labelled particles, *in situ* instruments may be used to measure concentrations at specified locations. For all types of tracers, samples may be taken at recorded

locations and the concentration determined, either on-site or in the laboratory. The latter is usually used for bacterial spores and bacteriophages. In order to obtain comprehensive information on an area, releases will need to be undertaken on different occasions from a range of locations and under differing tidal and meteorological conditions. However, the release location is often confined to one or a small number of the principal sources of contamination and the study conditions may be confined to those that prevail in the area or the worst case.

vi. ***Complex hydrodynamic modelling*** – this involves the use of either an off-the-shelf or a custom 3D modelling package with appropriate input of depth and boundary state tidal information. The model should be able to replicate the effects of wind of different direction and strengths and also the effect of stratification and density driven currents. Where appropriate to the location, it should also be able to take account of drying areas. A particle tracking model may be superimposed on the hydrodynamic output in order to determine the fate of one or more contaminants from one or more sources over a range of conditions.

Explanation

The hydrodynamics of an area is the way that the water moves around it. This may be influenced by oceanic currents as well as more local tidal, wind and density driven currents. Those currents may be modified in both direction and speed due to the presence of islands, promontories, and other land forms. The hydrodynamics will ultimately influence the direction that contamination will be taken from a source and the distance that the contaminant will travel. This will also be influenced by dispersion and dilution.

Some areas include islands that are connected by a promontory to the mainland at low tide (sometimes only at low spring tide). In such situations, the path taken by currents, and any contamination, may differ markedly between high tide and low tide.

Modelling or tracer studies may already have been undertaken in the area, for example as part of the supporting evidence for a sewage improvement scheme. In such cases, the output s may be used for the growing area assessment if the conditions and locations used in the study(ies) were appropriate. Where models have been constructed, but the locations or conditions are not appropriate to the growing area assessment, it may be possible to gain access to the model in order to rerun appropriate scenarios.

Stratification

Recommendation

Determine whether stratification occurs in the area.

Other considerations

Either obtain this from existing information or data from previous studies undertaken in the area or determine it by measuring salinity and temperature at a number of locations at specified depths (or continuously with depth) using a

conductivity/temperature/depth (CTD) meter. Stratification may vary with season and meteorological conditions and so it may be necessary to conduct such measurements on a number of occasions.

Explanation

Stratification of water bodies is the formation of distinctly separate layers, usually due to differences in salinity or temperature. The upper layer is less dense than the lower layer. Contaminants introduced into one layer may then be constrained within that layer, reducing the vertical spread of the contaminant, reducing the effective dilution from that which would occur if the contaminant was distributed throughout the depth, and potentially increasing the distance over which a significant concentration of the contaminant may be detected.

Stratification may also be important in some areas with respect to harmful algal blooms as the occurrence of the algae may be predominantly confined to certain depths within the water column.

In some water bodies, inversion may occur at one or more times of the year. In an inversion, the bottom stratified layer rises to the surface and displaces the layer that was there. This markedly changes the nature of the water body and will cause mixing and possibly re-suspension of sediment contaminants.

3.1.2.4 **Meteorology**

Rainfall/precipitation

Recommendation

Identify the general precipitation patterns affecting a growing area and its impacting catchments, both with respect to general variation in amounts of rainfall through the year and also the frequency and intensity of higher rainfall events.

Other considerations

It is important to determine the geographical relationship of the nearest rain gauge to the growing area. If necessary, it may be necessary to place a rain gauge within the assessment area to ensure that the data is applicable to the area. Where more than one catchment may impact on a growing area, potential differences in rainfall patterns and effects between catchments should be considered.

Reporting practices relating to total precipitation vary markedly. Snowfall may be converted to a rainfall equivalent and combined with rainfall values to give total precipitation, the two values may be reported separately, or rainfall only values may be given. Information on the specific approach may be available in the metadata for an individual weather station. It is important to know as the effects of rainfall on contaminant levels in the aquatic environment may be seen from a small number of hours (or sooner) to days whereas snow tends to have such effects when melt occurs. Snowmelt may be months after the snow has fallen.

Explanation

Rainfall and/or snow or ice melt may cause increased flows in watercourses and may result in spillages from sewerage networks and retention tanks associated with sewage treatment works, reduce the effectiveness of sewage treatment by increasing flows, cause spillages from improperly sized slurry storage tanks and wash faecal material off land. Increased flows in watercourses may also re-suspend particulate material which may have associated contaminants.

Wind

Recommendation

Information should therefore be obtained on predominant wind direction(s) and how wind direction and speed varies with season.

Other considerations

The vicinity of the wind gauge to the growing area is important as the local topography will affect the wind direction and speed. Where an established wind gauge is not in a location appropriate to the assessment area, it may be necessary to place a wind gauge within the locality to ensure that the data is applicable to the area.

In areas that have weak tidal currents (usually associated with low tidal amplitudes) wind may be the most dominant factor in the transport dispersion, and dilution of pollution sources.

Explanation

Winds primarily affect the currents in an area, and thus whether, and to what extent, contaminants from a specific source(s) reach the area where the bivalves are growing. However, winds can also enhance mixing of contaminants within the water column For example, in areas with a large fetch[21], wind driven swell and chop may cause re-suspension of sediment. In areas with weak tidal currents, wind-driven currents may predominate. Moderate to strong winds may also modify the direction taken by the plume of a discharge from a watercourse (e.g. a plume of potentially contaminated fresh or partly saline water emanating from the mouth of a river).

Severe storms

Recommendation

The occurrence, nature and frequency of such storm events should be determined together with the historical effects and whether any effective warning systems are in place.

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[21]   Fetch is the distance traveled by wind or waves across open water.

Explanation

Storms often give the combined effects of severe wind and high rainfall with more extreme effects than those seen during normal bad weather but with destruction leading to potential rupture of sewerage systems, breakdown of sewage works and pumping stations and chemical contamination due to damage to industrial units, storage systems and piping. In addition, run-off from urban areas land may be extreme and the amount of suspended material and re-suspension in watercourses and the estuarine and marine environments may be extreme. Such events may result in marked contamination with microbiological and chemical hazards. The information will determine whether the storms need to be included in the expected or unexpected event management plans.

Sunshine

Recommendation

Obtain data on mean daily sunshine amounts and intensity through the year.

Other considerations

The vicinity of any sunshine recording equipment to the assessment area is important as local microclimates may affect the amount of cloud cover and thus the amount and intensity of sunshine. However, local sunshine recorders may only provide daily sunshine amounts and subsidiary regional or national data may need to be used to estimate intensity through the year.

Explanation

Sunshine levels affect the amount of UV inactivation of microbiological contaminants. However, the intensity of UV declines with water depth and is also affect by the amount of suspended and dissolved material in the water. In addition, some bacteria that have been exposed to UV during a sewage treatment process may be reactivated by exposure to visible light. It is therefore difficult to predict effects and it may not be possible to assess information on sunshine levels without local data on the effects on specific microbes in the natural environment.

3.1.2.5 Seawater temperature and salinity

Recommendation

Seasonal variability in water temperature and salinity in the assessment area should be determined. Year to year variability in these factors should also be considered. This should include the effects of known climatic events such as *El Niño* and *La Niña*.

Other considerations

Where variations in temperature and/or salinity are known to occur in the assessment area, data should be obtained at the appropriate scale. In addition, variations in depth

through an area (including intertidal areas), the influence of large watercourses or industrial discharges and stratification (including turnover of stratified layers) may mean that temperature and/or salinity may vary significantly within an assessment area.

Explanation

Seawater temperature and salinity have an influence on the survivability of faecal indicator bacteria and pathogens. Marine vibrios will multiply in the marine environment at suitable conditions of temperature and salinity.

In general, in areas where *V. parahaemolyticus* and *V. vulnificus* have been identified as a potential hazard, the following conditions have been identified as usually being associated with a lower risk of infection.

> *V. parahaemolyticus:* water temperature <15°C or salinity >35 ppt
>
> *V. vulnificus:* water temperature <20°C or salinity >30 ppt
>
> (CAC, 2010a).

These factors therefore influence risk periods during the year and such periods will vary with local climate and environmental conditions. In addition, local factors may cause small-scale effects within a single growing area and these factors should be considered if the risk from pathogenic vibrios has been identified as being moderate or high. However, information is limited on variation of pathogenic vibrio concentrations with small scale variations in temperature and salinity within growing areas. Air temperature may also affect the concentration of vibrios pre-harvest in bivalves grown intertidally: if vibrios have been identified as a hazard for areas where such harvest occurs, information should be obtained on diurnal temperature variation through the year (Nordstrom *et al.*, 2004).

In the presence of sufficient nutrients, faecal indicator bacteria and some other bacterial pathogens (including some *Salmonella* strains) may proliferate in the marine environment at temperatures encountered in tropical countries. This effect will be greater in estuarine and brackish waters.

3.2   SHORELINE SURVEY

The shoreline survey constitutes a physical inventory of potential and actual sources of pollution relevant to the potential hazards and impacting factors identified during the risk profile and other potential sources relevant to those hazards that are observed during the survey.

3.2.1   PLANNING

Recommendation and Explanation

Sufficient time and resource should be applied to planning of a shoreline survey prior to going into the field. The following should be taken into account:

> Health and Safety – fieldwork can be dangerous and a risk assessment should be undertaken that, in addition to national and organizational requirements, takes into account the specific characteristics of the assessment area and the type of work to be undertaken. Good planning of all elements of the shoreline survey assists safe working.

> Access – access may be restricted by physical constraints (e.g. cliffs, large watercourse, muddy areas) or legal restrictions (e.g. inability to access private property, closed areas associated with military firing ranges). In some countries, environmental regulators or local authority officials may have more rights to enter relevant properties than food safety officials and in these cases a joint survey team may prove of value. Such an approach may also broaden the expertise available to the survey team.

> Tides – in areas where tides are of consequence, these may affect not only access but also what can be observed. Low spring tides expose more of the foreshore and potentially allow more features, such as discharge pipes, to be observed, recorded and sampled. However, where boats are used for all or part of the survey, for example to record the location of aquaculture resource or to record features on the shoreline where coastal access is not feasible, access may be limited by the depth of water needed to operate the vessel safely.

> Daylight – is necessary for safe working and to record target features. The correct tidal conditions for undertaking work may not occur at times that yield a reasonable amount of daylight survey time. In such cases, planning of the survey needs to strike a balance between the various requirements. In moderate and high latitudes, the daylight period during winter may be too short, especially when other factors are considered.

> Weather – may have a significant effect on safe working, especially if boats are to be used. It also significantly affects some items that may targeted for recording and/or sampling. For example, combined sewer overflows, other storm discharges and watercourses may not be flowing during dry weather. At the same time, after heavy rainfall/above average conditions significantly greater resource may be required to effectively record and/or sample all of the sources that are flowing. Resource constraints may prevent targeting a shoreline survey at specific weather conditions although this may be done when the survey is part of an investigation into unexplained high faecal indicator results or pathogen contamination. Although wet weather surveys will often be preferable given that more potential sources will be flowing, dry weather surveys may be useful in distinguishing or finding sewage discharges or illicit connections to storm drains that might otherwise be masked under wet weather conditions.

> Seasonality. This can affect:

  > recreational activities – boating activity, use of holiday homes;
  > farming practices – stock movements/concentrations, e.g. for annual shearing of sheep or drafting off of livestock to go to abattoir, concentrate many animals in one place; and

> wildlife –migration and activity.

It may therefore be necessary to undertake parts of, or even the whole shoreline survey, at different times of the year in order to capture all components.

> Available information – the information and data obtained for the risk profile and the desk-based parts of the growing area assessment should be reviewed in order to identify items for targeting during the shoreline survey. For example, this may be confirmation of the location of, or visits to, certain sewage works or livestock operations, or items of information where the location, function or intensity of operation may not be clear (e.g. conflicting locations given by two sources for the same asset, outfall pipe no longer in use due to changes in sewer network).

An example shoreline survey checklist to assist with the planning of a shoreline survey is given in Annex 4. It is recommended that a written plan is prepared in order to help ensure that all aspects are covered and so that all staff involved in the fieldwork know what needs to be done. An example template is given in Annex 5. However, it must be emphasized that it may be necessary to modify the plan during the work due to the situation in the area being different to that expected, or for health and safety reasons.

3.2.2   CONDUCT OF SHORELINE SURVEY

Recommendation

The following information should be sought and recorded during the shoreline survey:

> location of the bivalve mollusc resource;

> location of sewage or other waste water treatment or discharge systems (including discharges from abattoirs, food-processing factories, industrial plants);

> visual or other physical evidence of malfunction of such systems, including septic tanks, effluent soakaways, seeps and/or drainages, etc;

> available evidence of content or flow (e.g. if access to relevant meters is available);

> occurrence of direct human defaecation to land;

> agricultural activities (farm animals, slurry storage sites, sludge and slurry application, presence of any amelioration measures (farm animal buffer zones, farm drainage diversion);

> wild animal and bird populations, especially where concentrated in specific areas (and in relation to pollution sources such as landfills where pollutants may be transmitted by birds to the growing areas);

> sea traffic, ballast water, recreational boating activity and seasonality thereof; and

> water courses entering the marine or estuarine environments.

Photographs should be taken of each significant item (potential pollution source, measurement or sampling site, etc.) made during the shoreline survey in order to provide a fuller record and to allow further interpretation during the Growing Area Assessment.

During the shoreline survey, samples relevant to the hazards identified in risk profile should be collected and analysed. These may include samples of bivalves, seawater, discharges and watercourses. Sampling, sample transport and analysis procedures should be defined in advance and should conform to the recommendations given in Section 4.3. The delay between taking a sample and delivering that sample to the laboratory may be greater for a shoreline survey than for routine monitoring. Samples should be placed under appropriate transport conditions as soon as possible after collection and those conditions maintained until delivery to the laboratory. For samples for bacteriological testing, this means that they will need to be placed under temperature control during the survey itself rather than waiting until return to transport (for surveys by foot) or port (for surveys by boat).

A written report of the shoreline survey should be prepared and included in the overall Growing Area Assessment report, either as a section of that report or as an annex. A shoreline survey report template is given in Annex 6.

Other considerations

The recommended items given above largely relate to hazards of faecal origin although industrial activity and effluents, together with some aspects of agricultural and ship/boat activity are also relevant to chemical contaminants. It is important that if other types of hazard have been identified during the risk profile stage, relevant targeting of additional information and sampling is included in the shoreline survey plan.

The shoreline survey report should normally include a summary of the principle observations together with record of the observations made during the survey, the results of any samples taken, and photographs of key observations.

Video recording may usefully supplement the use of photographs in providing a fuller record and assisting later interpretation.

Explanation

The shoreline survey provides an opportunity to verify data obtained from other bodies during the desk phase of the growing area assessment. It also provides the opportunity to identify other infrastructure or characteristics of an assessment area, relevant to the hazards under consideration that were not included in information and data obtained during the desk phase. For some countries, regions or areas, the shoreline survey may provide the most important, or even only, source of data for some aspects of the growing area assessment. In such cases, it is necessary to devote a proportionally larger amount of time and resource to the shoreline survey in terms of the recording and sampling that is undertaken.

3.3   INDICATOR/HAZARD SURVEY

Recommendation

Determine whether an indicator/hazard survey is necessary as part of the Growing Area Assessment and, if so, plan and undertake the survey with appropriate sampling and laboratory analysis. Sampling, sample transport and analysis procedures should be defined in advance and should conform to the recommendations given in Section 4.3.

Other considerations

It is necessary to identify the sampling locations on the basis of the information and data collected for the desk phase of the Growing Area Assessment. These locations may either be intended to reflect expected worst case occurrence/concentrations or to show variation in occurrence/concentration across an area.

For a general microbiological survey, it is preferable for samples to be taken on at least three occasions, each separated by a period of at least two weeks, in order to provide some information on temporal variability. The number of sampling occasions for other indicators/hazards should be determined on the basis as to whether such temporal variability is expected. It may be necessary to specifically target sampling occasions, e.g. during a specific season, after operation of intermittent sewage overflows, etc.

Where possible, it is preferable for one of the sampling occasions to occur at the time of the shoreline survey so that the results may be related to the conditions at the time of the shoreline survey and to any observations and results from potential sources that are obtained from that survey.

Explanation

A survey of the assessment area with respect to the presence (and, where relevant, concentration) of indicators (e.g. faecal indicator bacteria and/or MSC) and/or specific hazards (e.g. norovirus, hepatitis A, target chemical contaminants) may be undertaken in support of the Growing Area Assessment. This will normally involve sampling water and/or bivalve molluscs at a number of locations on one or more occasions. Whichever matrix is used is for the purpose of classification (water or molluscs), it is useful to target additional monitoring of indicators and hazards at the bivalve mollusc themselves.

Sampling of indicators (faecal indicator bacteria or MSC) and/or hazards may also be undertaken to support an assessment of treatment efficiency of a sewage treatment works or a chemical waste reduction process. This will require a number of paired samples to be taken at influent and effluent locations in order to obtain an average estimate of reduction through the process. It must not be assumed that the results obtained from influent and effluent samples taken at approximately the same time relate to the treatment efficiency at that moment as there is normally a significant retention time within the treatment system. This means that the effluent at any specific moment in time actually relates to influent entering the works some time previously.

3.4 DATA ANALYSIS AND ASSESSMENT

The analysis comprises a review of the risk profile, the additional information and data gathered for the purposes of the growing area assessment (both on contamination sources and environmental factors) and the shoreline survey. The analysis is the detailed examination of those items of information and data while the assessment is the process by which the outcomes of the Growing Area Assessment (see Section 3.5) are identified based on that analysis.

3.4.1 ANALYTICAL APPROACHES

Recommendation

Analytical approaches may be:

> Descriptive/qualitative;

> Semi-quantitative; or

> Quantitative.

These are described in Sections 3.4.1.1 to 3.4.1.3.

Normally, the simplest approach should be used that is appropriate to the characteristics of the growing area, the data available and the hazard(s) being considered (and thus fit for purpose). It is not possible to recommend a level without considering those specifics.

Other considerations

In general, the best approach is to start with the descriptive/qualitative approach and to proceed to the other levels as necessary to properly evaluate the hazard. The assessment level is dependent on the appropriate data being available. For example, it is not possible to undertake a fully quantitative assessment of the impact of sewage discharges if quantitative data is available on the discharges but insufficient data is available on the hydrodynamics of the area. In such cases, it may be possible to undertake a semi-quantitative assessment including the use of a simple dilution model.

Unless specifically allowed for in the assessment approach, a descriptive or semi-quantitative assessment may result in a less conservative outcome than a fully quantitative assessment. For example, this may be because the simpler approaches assume an impact based on the expected level of a contaminant in a discharge (e.g. the level authorized by the environmental regulator) whereas the fully quantitative approach may take into account actual concentrations recorded over time. Alternatively, the simpler approaches may assume uniform dispersion/dilution in all directions whereas the fully quantitative assessment may take into account concentration of the contaminant in a plume from a discharge. However, this depends on the fully quantitative assessment properly representing all of the factors relevant to the occurrence of the contamination in the source and dispersion/dilution in the environment.

In some situations, it may be appropriate to use a descriptive or semi-quantitative approach for the general assessment but a fully quantitative assessment for one specific component. This component may be a large sewage discharge that is either considered to be the predominant source of faecal contamination in the assessment area or may be subject to ongoing maintenance problems at the associated treatment works (assuming that the discharge relates to treated effluent). Alternatively, the fully quantitative component may relate to a specific hazard, such as a chemical or radiological contaminant that is known to have a source (or sources) within the assessment area and where a more detailed assessment of the potential impact on the bivalve resource is required. This will require the necessary level of data being available on the sources of the contaminant and the hydrodynamics of the area.

Explanation

The intention is to reflect the geographical distribution (where relevant, in three dimensions) of the risks from each hazard across the assessment area and the variability that may arise due to variation at source and to environmental and biological factors. It is therefore the aim to reflect the inter-relationship of all of those factors.

3.4.1.1 **Descriptive/qualitative**

Recommendation

The principle sources of contamination relating to each hazard, or group of hazards, is determined based on expert judgment. The locations and nature of these inputs should then be identified on a map showing the general characteristics of the assessment area and location of bivalve mollusc resource(s). Where possible, the predominant current flow(s) in the area should also be identified on the map. The assessment should then be based on a judgment as to the likely combined impact from the various sources considering the information on current flow(s).

Other considerations

For enteric pathogens (and thus consideration of implications for faecal indicators), the sources will be principal sewage works, marinas and large clusters of moorings, specific intertidal areas used by large numbers of seabirds and waders, and a large cattle feedlot might be considered the most important faecal contributions to an assessment area (it must be emphasized that the identified sources would differ from area to area). The approach is undertaken separately for each hazard, or group of hazards, under consideration. Where sources are not relevant to a hazard, other factors relevant to the hazard should be addressed in the same way. Such factors may or may not have a geographical component. If not, the map element of this approach will be excluded for consideration of that specific hazard.

An example map for a qualitative assessment is shown in Figure 3.3. The sources have been shown in relatively close proximity to the bivalve resource for illustrative purposes.

Explanation

This approach is the simplest means of assessment but may be the appropriate means because the principal sources of the relevant hazards are evident as is their impact across the assessment area. Use of this approach may be dictated by the lack of data on which to undertake a more complex assessment.

FIGURE 3.3 **EXAMPLE MAP FOR A QUALITATIVE ASSESSMENT**

ESTIMATED TRANSPORT DISTANCE
over a single spring tide

WATERCOURSES

WILD BIRD SITES

SHEEP FARMS

CATTLE FARMS

INTERMITTENT SEWAGE DISCHARGES

CONTINOUS SEWAGE DISCHARGES
by dry weather flow (m3/day)

○ 500 to 2 000

○ 0 to 500

by treatment level

■ secondary

▲ tertiary

BIVALVE ROSOURCE

SEA

LAND
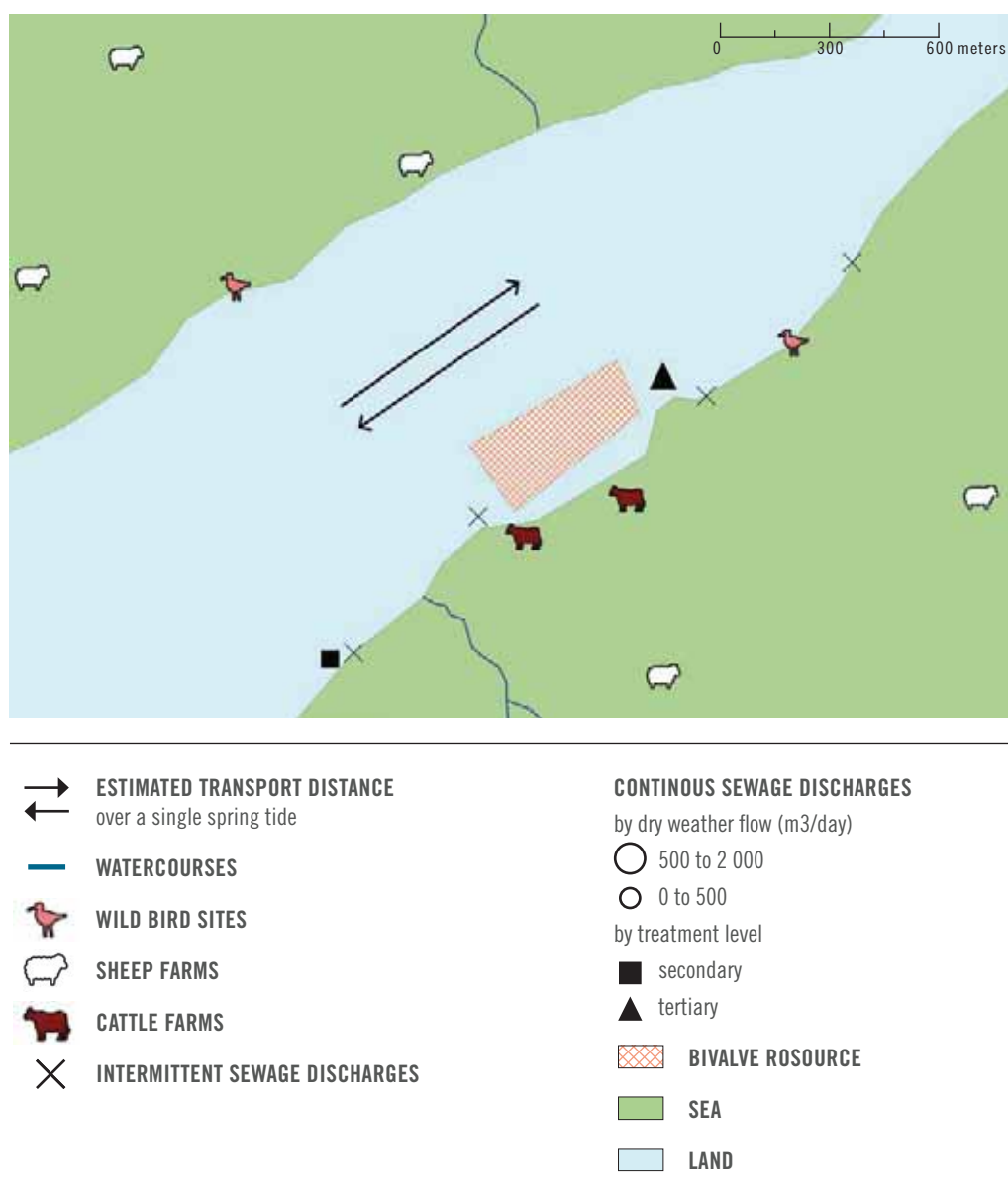
3.4.1.2 **Semi-quantitative**

Recommendation

Rank the identified sources of contamination relating to each hazard, or group of hazards, by their potential contribution to the assessment area (preferably, to the bivalve resource). Give each source a loading score, with those with the greatest potential contribution being given the highest score. Also give each source an occurrence score, with the lowest positive score occurring infrequently and the highest being a continuous input (e.g. a continuous sewage discharge). Finally assign each source a separate score in relation to its proximity to one or more assessment points (if there is more than one assessment point, it is simplest if a separate ranking table is prepared for each), with the points preferably located on the bivalve resource. Determine the impact of each source at the assessment point as the product of the contribution score and the proximity score. Sum the impact scores for each source to give an overall score for each assessment point. The same approach can be used for a subset of faecal inputs (e.g. only those relating to human inputs) or for sources of other hazards.

Other considerations

Where information is available on the predominant currents, this is used to determine an impacting proximity (essentially the inverse of distance), rather than relating this to a simple geographical measurement. Where this is done for a number of assessment points, it gives a semi-quantitative assessment of the impact from several sources across an area or resource. The whole procedure is undertaken separately for each hazard or group of hazards. The scores are usually shown in table form (see Table 3.1). However, it is also useful to show the outputs for each source/ assessment point on a map of the area, with the size of a symbol for a source being proportional to the contribution score, and the likely transport distances shown by arrows, annotated with the proximity score. The size of the symbol for each assessment point can be shown proportional to the combined score (The map given in Figure 3.4 only shows the location of the contamination sources referred to in Table 3.1 and not the other characteristics such as size proportional to contribution score or likely transport distance.).

Loadings from sources may be ranked from 0 to 4 with zero representing sources with no significant loading of the hazard and 1 to 4 representing the 1st, 2nd, 3rd and 4th quartile loading ranges respectively. The quartiles may be determined may be determined for each assessment area, from the data available for the sources in that area. However, greater comparison between assessments will be achieved if this is determined over the range of sources impacting all assessment areas within the programme.

The occurrence score reflects the proportion of time (not just the number of spills for a storm discharge, for example) that the source impacts on the assessment area. It may also be ranked from 0 to 4. A zero value will reflect no output and 4 will represent a continuous impact. The values from 1 to 3 will present increasing proportions of time for which the source impacts on the assessment area.

FIGURE 3.4 **MAP OF SEMI-QUANTITATIVE EXAMPLE AREA**
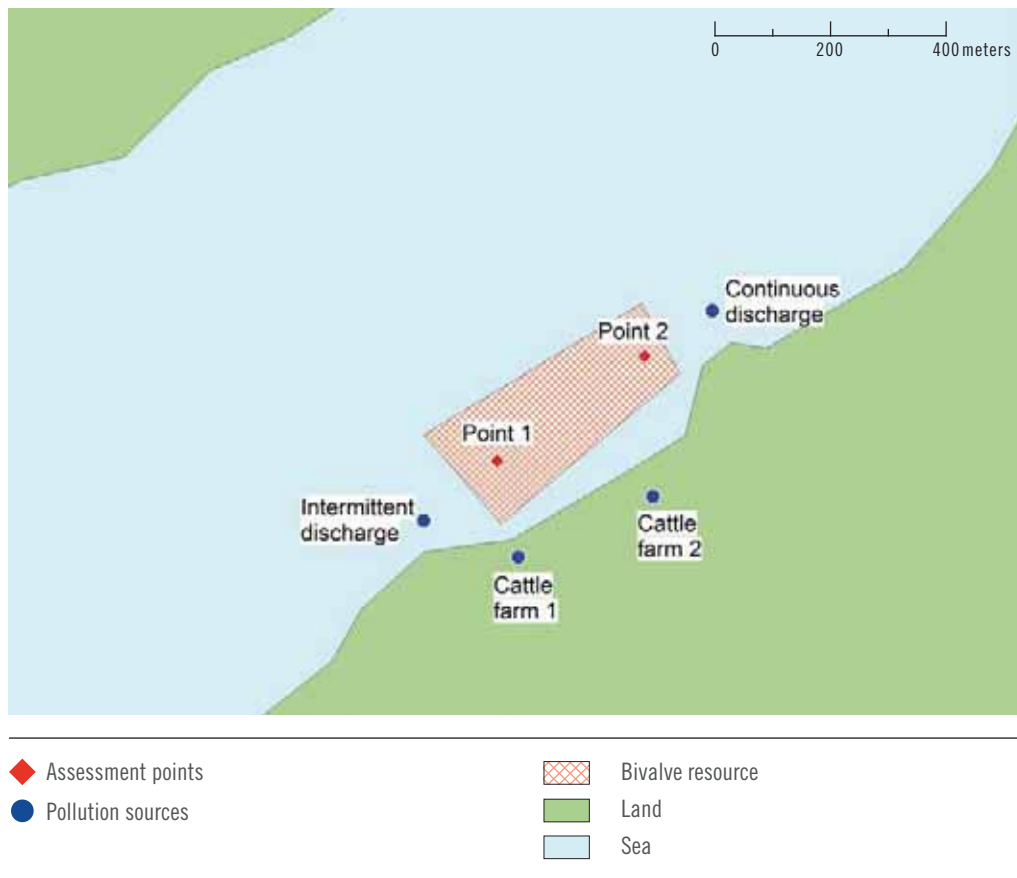
Where data allows, this may be determined on the basis of the average number of hours a year that an intermittent source impacts and assessing this as a proportion of the total hours in a year.

No output	0
Up to 2190 hours per year	1
2190 to 5380 hours per year	2
5380 to 6570 hours per year	3
More than 6570 hours per year	4

For example, most intermittent sewage discharges would be given a frequency score of 1 as the combined number of hours spilling would be less than 2190 hours per year (a full year is 8760 hours, ignoring leap years). However, the number of hours are only given to indicate the principles. In many cases that level of detail will not be available and the score will be based on a rough estimate.

Proximity may be scored from 0 to 4 with zero being assigned to those sources are that beyond the maximum hazard transport distance determined during the hydrographic element of the Growing Area Assessment. The maximum transport distance is then divided into four equal distances and these are then represented

by 1 to 4 in decreasing distance from the assessment point (thus 4 will be given to those sources in the vicinity of the assessment point). A score of 0 would apply to any sources beyond the maximum hazard transport distance.

For hazards that are not related to specific sources, a ranking is given to each assessment point on the basis of the factors that affect the occurrence, concentration or risk of that hazard.

Explanation

The semi-quantitative approach is to be preferred to the qualitative approach, where data allows, as it allows some comparison of the potential impact of sources with respect to the hazards. While requiring some additional resource compared to the qualitative approach, this is not great in comparison with a fully quantitative assessment.

TABLE 3.1   **RANKING METHOD FOR ESTIMATING IMPACTS**

ASSESSMENT POINT 1

SOURCE	RELATIVE LOADING	OCCURRENCE	PROXIMITY	IMPACT
Continuous discharge	2	3	3	18
Intermittent discharge	3	1	5	15
Cattle farm 1	1	1	5	5
Cattle farm 2	1	2	4	8
Total				41

ASSESSMENT POINT 2

SOURCE	RELATIVE LOADING	OCCURRENCE	PROXIMITY	IMPACT
Continuous discharge	2	3	3	18
Intermittent discharge	3	1	3	9
Cattle farm 1	1	1	3	3
Cattle farm 2	1	2	4	8
Total				38

- Each aspect (relative loading, occurrence, proximity) has been a score in the range of 1 to 5.given a score in the range 0 to 5.

- Proximity is ranked inverse to waterborne distance

- The continuous discharge has been given an occurrence score of 3 as it only impacts across the resource when the tidal current is flowing to the west south west.

3.4.1.3 Quantitative assessment

There are three separate aspects to a fully quantitative assessment. One is calculation or of the contribution of each source for the hazard, or group of hazards, in question, as this applies at the point where it enters the assessment area. The second is a calculation of the transport of the hazard within the area, together with its dilution and dispersion during that transport. The third is a calculation of the impact, or otherwise (i.e. no expected impact), of each source at specific locations within the assessment area.

3.4.1.3.1 *Quantitative source estimation*

Recommendation

Where possible, estimate the contribution of the various sources relevant to the hazard using a common metric. For example, the potential contribution of enteric pathogens by different sources may be compared by estimating the loading of faecal coliforms or *E. coli* (normally expressed as bacteria per day) associated with that source, be that a sewage discharge, a watercourse or an animal feedlot.

Other considerations

The variability in hazard content and (where relevant) volume rate of input of the sources should be ascertained and used to determine the likely range in contribution to the environment.

In addition, any uncertainties relating to the data should be estimated as this may significantly affect the resulting assessment. Such uncertainties may include lack of data on key aspects such as loading, and frequency and timing of discharge, with the use of estimates (e.g. obtained for similar types of discharge from the scientific literature or other sources).

Explanation

Quantifying the contributions of the sources in this manner gives an estimate of the importance of their contribution to the area. It also allows the contribution from different sources to be compared.

3.4.1.3.2 *Quantitative transport estimation*

Recommendation

Use one of the following methods to determine transport, or its effect:

> simple calculations of dilution in the receiving water based on volume (from area and depth);

> simple calculations of dilution in the receiving water based on salinity measurements. This is only relevant when the source of salinity reduction is due to, or related to, the source of contamination;

> tidal stream (or other current-related) estimations of contaminant transport distance;

> dye or other tracer studies; or

> hydrodynamic modelling.

Other considerations

Proceeding from a simple dilution estimation to full hydrodynamic modelling has implications for the type and extent of data that is needed and for the amount of

resource in terms of expertise, time and cost that has to be applied. In general, a more complicated approach is used where there is reason to believe that simpler approaches will, or have, yielded results that significantly differ from the real situation and where either:

> this would result in a significant underestimate of the impact of a hazard across the bivalve resource (and thus have significant potential outcomes for public health); or

> a more accurate estimation would allow the utilization of bivalve resource that would otherwise be included in a closed area.

Each of the approaches has its own advantages and disadvantages. Simple dilution estimates, especially when based on the volume of the receiving water, do not reflect the effect of transport pathways within the assessment area and assume even dispersion of the contaminant through the receiving water. The amount of dilution at any point in the assessment area will often be an overestimate: however, if the direction of currents in an area means that contamination is not taken towards a point then the method may produce an underestimate of dilution. Tidal stream-based estimates give a simple method of estimating contaminant transport direction and distance. Dye tracking and hydrodynamic modelling approaches both involve the application of significantly more resource but should overcome the drawbacks of simple dilution estimation. Dye tracking studies have the advantage of reflecting the real world situation but resource limitations usually mean that they can only be undertaken for a small number of environmental conditions (e.g. in relation to wind direction and strength and rainfall). In addition, a separate study will need to be undertaken for each potentially significant source. Hydrodynamic modelling can potentially reflect the impact of several sources under a range of environmental conditions: however, complex models are usually needed to approximate the real world and a large amount of data is usually needed to both set up and validate the models.

Each type of hydrodynamic assessment approach will involve uncertainties with respect to the underlying data used in the approach and how well the approach chosen represents the actual transport, dilution and dispersion of the sources of contamination with respect to the assessment area and the bivalve resource. Selection of the approach should take into account the likely magnitude of the variabilities and uncertainties involved.

Annexes 7, 8 and 9 give the key considerations for undertaking drogue studies, hydrodynamic modelling, and dye studies respectively.

Explanation

Quantitative transport estimation will usually give a much better evaluation of the fate of contaminants within an assessment area than will be obtained using simpler methods. However, it requires the application of relevant expertise and requires appropriate quality control and validation. Estimates may be restricted to certain

conditions and it may still be necessary to use expert judgment to extrapolate the outcomes to other conditions.

3.4.1.3.3 *Quantitative impact estimation*

Recommendation

Estimate the quantitative impact of the hazard(s) or indicator at one or more assessment points, or across the assessment area (primarily concentrating on the bivalve resource). The impact is usually determined as the average concentration and variability of the hazard(s)/indicator. This should be undertaken for a range of conditions (e.g. tidal, meteorological) relevant to the assessment area and the hazard(s)/indicator. Those conditions should include those that prevail in the area (e.g. predominant wind direction and speeds) and those that may lead to greatest impact.

Other considerations

Where transport has been calculated based on dilution estimates or tracer studies, the impact at the assessment point(s) is calculated directly as the reduction in concentration of each source due to the calculated dilution. Where more than one source may impact at the point(s), the contribution of each source is determined and the final impact determined by summing these values.

Where hydrodynamic modelling has been undertaken, this may be used to determine dilution and the approach outline above used. More usually, a particle transport model will be used that allows the source input concentrations to be superimposed on the output of the hydrodynamic model. This will then provide calculated particle (i.e. hazard or indicator) concentrations over the modelled area.

If decay of the hazard(s)/indicator occurs in the environment, an estimate of this decay rate may be applied to the estimate of the concentration at the assessment point(s). However, care must be taken to ensure that any decay rate values are appropriate to the assessment area, and the pathogen modelled (e.g. lower or no decay values may be more appropriate for viruses). Local variables affecting decay rates are the degree of insolation and turbidity, salinity and temperature of the water. Where relevant decay rates are low relative to dilution and dispersion, their addition to the assessment will not yield any significant advantage.

Build-up of concentrations of hazards/indicators may occur over successive tidal cycles. The resulting concentrations may be markedly higher than those determined over a single tidal cycle. The magnitude of the effect varies between locations depending on the hydrodynamics. Tracer studies and modelling approaches may be designed to allow the estimation of such a build-up.

The variability and uncertainty of the impact of an indicator or hazard over the area of the bivalve resource will be an interaction of the variabilities and uncertainties associated with the source (where relevant) and transport estimations. These should be described as fully as possible.

Explanation

A quantitative impact assessment will provide the best estimate of the impact of contaminating sources across an assessment area, assuming good base data and proper validation. It will also contribute directly to the determination of buffer zones (see Section 5.7). However, the resource required is great and the approach is usually reserved for areas where investigations have shown that a greater level of assessment is required.

It is possible to undertake mixed level assessments. For example, where quantitative transport estimates are available from other studies, these may be combined with qualitative or semi-quantitative assessment of sources.

3.4.2 VALIDATION OF THE ASSESSMENT

Recommendation

Quantitative assessment methods should include appropriate validation of the data and outputs. Additionally, to those procedures, validation of the assessment itself (qualitative, semi-quantitative or quantitative) should be undertaken.

At the simplest level, the validation of the outcomes of the assessment comprises a simple check as to whether they appear sensible with respect to experience from similar areas and other information on the assessment area itself.

Other considerations

Additionally, more specific checks may be undertaken, for example if there are sample results for the area relating to the specific hazard(s) under consideration. These may be from previous monitoring for bivalve mollusc sanitation purposes, from other water quality programmes or from samples taken during the shoreline survey. It is important that any data used for validation is separate from that used to support the assessment itself. Care must also be taken in assessing such data with respect to the matrix sampled, location of sampling and number of results available as these factors will affect the relevance and use of the results. If there is an apparent conflict between any monitoring data and the assessment, this should trigger a review of the assessment rather than overriding the assessment itself based on the data.

The validation should consider the associated variabilities and uncertainties included in the assessment. This part of the validation process may identify that the estimates of variability and uncertainty were too large and thus may be reduced. At the same time, it may identify that the estimates were too small and thus need to be increased for the assessment to be valid.

Explanation

Errors in the data used for the assessment or erroneous assumptions made during the assessment process may result in wrong conclusions being drawn from the

assessment process. All levels of assessment may be subject to these problems, including fully quantitative assessments. It is therefore important to undertake checks that the outcomes of the assessment reflect the physical situation in the area. In general, expert judgment of the outputs is simplified by the presentation of the key aspects (contamination sources, transport, impact), together with any key validation data, in visual form on a map of the area.

3.5 OUTCOMES

3.5.1 EXTENT OF THE CLASSIFIED GROWING AREA

Recommendation

Define the extent of the growing area that will be designated and classified. The extent of the growing area should:

> encompass as much of the identified bivalve mollusc resource, or intended aquaculture/wild harvest area, as possible; and

> exclude any areas around those sources that are deemed likely to be contain concentrations of a hazard that are not acceptable.

The growing area should be given a unique identifier and its boundaries defined by lines between points identified in latitude/longitude (preferably WGS84) and/or national grid format. It is useful for physical identification in the field if the points coincide with physical features (promontories, etc.). Navigation marks may also be used for this purpose but care needs to be taken with using these as they may occasionally be moved by the relevant authority. The growing area and the bounding co-ordinates should also be given on a map or chart in order to assist both industry and the control authority[22].

Other considerations

Where differential impacts are anticipated across an assessment area (i.e. different concentrations of a hazard in different parts of the area), and where parts of such an area may be managed differently, the resource or harvest area may be divided into two or more growing areas. For example, it may be anticipated that two parts of an assessment area may conform to different classification status. In such cases, these parts should be defined as separate growing areas, each with its own separate monitoring requirement. The final classification status of each area will still depend on both the growing area assessment and the outcomes of monitoring. Subdivided large areas, where justified by either being subject to different sources of pollution, or markedly different levels of hazards, has benefits for the industry in that, in the event of an event triggering management action, only a proportion of growing areas

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[22] The official body with responsibility for surveillance and enforcement of growing areas.

within a water body may be subject to closure or, if recall is required, only batches associated with the affected growing areas need to be considered. However, division of the resource or harvest area between growing areas should not be undertaken if the areas cannot be separately managed and enforced.

Areas outside classified growing areas should be considered unsuitable for harvest. However, it is useful for management activities if harvesting is explicitly prohibited in areas deemed likely to contain a hazard at concentrations markedly above those deemed acceptable. Such areas may include those around sewage outfalls or industrial discharges: these are often termed buffer zones. Areas designated as prohibited should also be given a unique identifier and be defined by lines between specified points. An example of the determination of a buffer zone is given in Annex 10.

Explanation

The primary outcome of the growing area assessment is to define the extent of the classified growing area in order to define the area that may be utilized for harvest and also to determine the area which monitoring must represent. It is important that the area is defined in a practical way in order to assist compliance with the boundaries by the industry and enforcement by the control authority.

3.5.2   RECOMMENDATIONS FOR PRIMARY MONITORING

Recommendation

The primary monitoring is normally targeted at the growing area itself, in order to assess the presence of indicators and/or hazards that may be present in the bivalve resource, and not at the potential or confirmed sources of contamination in the vicinity of the area.

The range of indicators and/or hazards included in primary monitoring should relate to the range of hazards identified as relevant during the Growing Area Risk Profile (see Sections 2.6 and 2.10). Where indicators are not expected to fully reflect the risk from one or more of the hazards that the indicator is intended to represent (e.g. faecal coliforms or *E. coli* and enteric viruses), supplementary monitoring of the hazard(s) themselves should be considered for inclusion in primary monitoring.

Recommended sampling plans should be produced for each hazard, or group of hazards, of concern, including any faecal indicator monitoring intended to reflect enteric pathogens (the faecal indicator monitoring will normally be used to support classification of the growing area(s), see Section 5). Considerations relating to the production of sampling plans for primary monitoring are given in Section 4.1 and the minimum recommended content for such sampling plans are given in Section 4.3.1.

Different matrices and/or sampling points may be identified for monitoring different hazards. In this case, it will usually be simpler to have separate sampling plans for each combination.

The location(s) together with the frequency and timing of sampling should properly the risk of the presence of the relevant hazard(s)/indicator(s) within the defined growing area.

Other considerations

Primary monitoring should cover those hazards identified as potentially significant to the growing area in the risk profile and also those required to be addressed by any applicable regulations. Indicators may be monitored in relation to one hazard or a group of hazards. For example, the level of risk from enteric pathogens is often represented by monitoring faecal indicator bacteria. However, any shortcomings in the indicator concerned with respect to a specific hazard should be addressed by supplementary monitoring for the hazard itself (or a suitable supplementary indicator).

Water is usually easier and cheaper to sample and analyse than bivalve molluscs. The results represent the water quality to which all bivalve species are reflected and thus obviate the need for sampling several different species. Analysis of water for a specific hazard may not be feasible or relevant. For example, the lower concentration of many hazards in water than in bivalve molluscs may mean that concentrations are near to, or below, the limit of detection or quantification of a laboratory method that can be applied in a routine laboratory.

Subsurface water samples are usually taken although it is possible to use specialized sampling devices to take water samples at specific depths. Subsurface water samples may not reflect the presence, or concentration, of a hazard at the specific location of the bivalves, especially if these are on lines or in the benthos (e.g. clams buried in the sediment).

In areas that are not well mixed and where contaminants from point sources (including the mouths of watercourses) are constrained in plumes that move with currents, the results from water samples may show marked variability both spatially and temporally. In such cases, monitoring the bivalves has the benefit of integrating contamination over time.

Sampling the bivalves themselves ensures that the presence, or concentration of hazard (or an indicator) that is measured is relevant to the resource to be harvested. However, this means that when there is more than one harvested species in a growing area, either each species has to be monitored separately for each hazard or indicator, or the use of an indicator species needs to be justified. A bivalve species may be used to reflect the hazard in other species if the presence or, or concentration of, the hazard or indicator in the indicator species is at least the same as, or greater than, than it the other species it is to represent. This should either be demonstrated by a robust study in the growing area or by a general robust study with confirmation by a smaller study that the situation applies in the growing area. It is essential to ensure that the geographical location, and position in the water column, of the indicator species is relevant to the other species. It should be noted that there is no universally applicable indicator species and the suitability for a bivalve species to represent

other species in an area needs to consider the applicability for each hazard being monitored. In addition, potential area-specific effects on the representativeness of a bivalve species for each hazard need to be taken into account.

The benefits of both matrices with respect to microbiological monitoring may be obtained by a base monitoring programme using water samples supplemented with more targeted monitoring of bivalve molluscs. There are two possible approaches with such a mixed programme:

> base water programme and targeted bivalve monitoring, with both undertaken for faecal indicator bacteria; or

> base water programme undertaken for faecal indicator bacteria and targeted bivalve monitoring, undertaken for additional indicators (e.g. MSC) and/or pathogens (e.g. norovirus, hepatitis A, but dependent on the hazards identified for the area).

Explanation

The primary monitoring is intended to supplement the Risk Profile and Growing Area Assessment to provide the basis for determination of the suitability of the growing area for classification and, where this is the case, to support determination of the initial classification category. A properly targeted sampling plan is necessary to properly show the presence (or absence) of the relevant hazard(s)/indicator(s): sampling at the wrong location or the wrong times may give a biased data set that does not represent the risk from the hazard(s).

3.5.3   RISK MANAGEMENT PLANNING

Recommendation

If the application of one or more conditional classification criteria (see Section 5.6) has been identified at this stage, then the criteria should be defined. Expected (which include the management of conditional classifications) and unexpected management plans should also be prepared (see Section 6).

Other considerations

Any conditional classification criteria, together with the expected and unexpected management plans should be provided to all stakeholders, including industry and the control authority.

The definition of conditional classification criteria may not be possible until the results of primary, or even ongoing, monitoring are available. In such cases, the definition of conditional classification criteria will be undertaken as part of the Growing Area Review process (see Section 7).

Explanation

The definition of any conditional classification criteria that may be applied and the preparation of expected and unexpected event management plans are an essential part of the assessment process and underpin the Growing Area Management process. Unexpected event management plans should be defined at this stage as these apply to any area. Some forms of expected events may be clear from the growing area assessment (e.g. widespread operation of storm-related discharges following heavy rainfall) and a plan may be established to manage these events. However, it may not be possible to define criteria for conditional classifications and to establish an associated management plan until after primary monitoring has been undertaken and an assessment undertaken of the results.

3.6 DOCUMENTATION OF GROWING AREA ASSESSMENT

Recommendation

The assessment, conclusions and recommendations from the Growing Area Assessment should be explicitly documented. There should be traceability from the underlying information and data through to the outcomes.

Other considerations

It is beneficial to summarize pertinent information and data relating to the outcomes of the Growing Area Assessment within a report that also contains those outcomes. Fuller data sets may be provided as annexes or as an electronic resource.

Explanation

It is important for both representatives of the responsible authority and stakeholders to be able to trace the outputs of the Growing Area Assessment to the underlying information and data and this is best achieved by formal documentation. The documentation also provides the basis for subsequent reviews.

CHAPTER 4
GROWING AREA MONITORING

Monitoring provides additional evidence for the presence of, and concentration of, indicators and/or specific hazards in a growing area. This supplements, but does not replace, the risk profile and growing area assessment elements of a programme as no monitoring programme can fully represent the risk of any individual hazard. Some of the reasons for this are:

> the hazard may not always be present in the potential source(s);

> even if always present, the concentration in the source(s) may vary with time (season, weather, time of day); or

> the hazard may only be present, or may only be present in high concentrations, after unexpected events.

Section 7.2.1 of the Codex Code of Practice envisages that monitoring may be based on sampling and testing of water and/or bivalve molluscs and/or sediments. This guidance considers monitoring based on sampling of water and/or bivalve molluscs. While the sampling and testing of sediments may be useful for specific investigations or longitudinal studies, it has not been used for routine growing area monitoring. The main reason is that sampling and testing sediments poses many of the difficulties and cost of using bivalves without directly giving information on the presence or concentration of a hazard at the start of the food chain.

This section will consider monitoring for faecal indicator organisms and microbial pathogens but not other hazards such as chemical contaminants and biotoxins.

4.1 PRIMARY MONITORING

4.1.1 PURPOSE

The primary monitoring undertaken in a growing area provides initial information on the level of faecal contamination, and other hazards, that are present and contributes to the classification of the growing area, and potentially other controls. The range of contaminants to be monitored, and the sampling plans for that monitoring, will be determined on the basis of the risk profile and growing area assessment. The monitoring may be undertaken to evaluate known issues identified during those elements or may be used to determine whether any issues actually exist with respect to potential hazards identified during the assessment process. The outcome of the primary monitoring may subsequently be used to modify and refine the range of contaminants and sampling plans for ongoing monitoring (see Section 4.2).

4.1.2 SELECTION OF SAMPLE MATRIX (WATER AND/OR BIVALVE MOLLUSCS)

The approach to the selection of matrix will differ between monitoring for faecal indicator bacteria and the monitoring for MSC and pathogens.

4.1.2.1 Faecal indicator bacteria (faecal coliforms or *E. coli*)

Recommendation

Determine whether water, bivalves or both are to be monitored in the growing area for the purpose of classification and ongoing assessment.

Other considerations

The matrix to be monitored may be dictated by existing regulations. Otherwise, preference for one matrix over another may be dictated by sampling and laboratory capabilities. If these constraints do not apply, it is recommended that the base classification be undertaken using water sampling with additional, more limited, sampling of bivalve molluscs (see Section 7.4).

Explanation

Section 7.2.1 of the Code of Practice envisages that classification monitoring may be based on *E. coli*/faecal coliforms or total coliforms. Total coliforms may be associated with a wide range of environmental sources, not necessarily of faecal origin, and thus this guidance is mainly confined to the use of *E. coli* or faecal coliforms.

Time series concentrations of faecal indicator bacteria in water may vary over an area due to the way that currents take contamination from a particular source to the sampling point. For the same reason, concentrations at a specific sampling point may vary markedly over short periods of time. This means that a relatively large number of samples are required to reflect spatial and temporal variability. However, these factors can be offset because the cost of sampling and testing water samples

is usually much lower than the costs for bivalve molluscs. Bivalve molluscs have the advantage that they reflect an integration of the prevailing concentration in the surrounding water over a period of time (from less than an hour to a few hours, depending on bivalve species).

Output from the Growing Area Assessment may enable targeting of monitoring to reduce variability. For example, in some areas targeting specific tidal conditions will reduce potential variability. However, it is important not to bias sampling towards conditions that produce low results when seeking to reduce variability.

4.1.2.2 Male-specific Coliphage (MSC)

Recommendation

The monitoring of MSC in bivalve molluscs should be considered when the risk profile has indicated that the risk from human enteric viruses is a significant risk and the growing area assessment has determined that this risk will not be adequately reflected by monitoring for faecal indicator bacteria. However, this will not be necessary if monitoring for all of the enteric viruses identified by the risk profile is undertaken. Testing of water and/or sewage samples for MSC should be considered as part of a suite of investigations of the efficiency of sewage treatment processes.

Other considerations

Analysis of bivalves for MSC may be undertaken on all of the samples taken for faecal indicator bacteria, or on a subset of these with respect to sampling site or frequency. If a subset of sampling sites is used, these should be targeted at the parts of the bivalve resource that are closest to sources of human faecal contamination, including watercourses that receive such contamination.

Studies of the relationship of the presence of MSC and norovirus have been undertaken in northern temperate climates (Goblick *et al.* 2011) but the relationship should be confirmed for use for other geographical locations and other viruses. Verification for individual growing areas could be achieved by undertaking parallel testing on samples taken for primary monitoring.

Annex 11 contains note on the application of male-specific coliphage monitoring. Annex 10 contains additional reference to the use of MSC in determining buffer zones.

Explanation

Monitoring for MSC may be used to better reflect viral risk than do faecal indicator bacteria, which are general indicators of recent faecal contamination. Within the context of a bivalve monitoring programme, samples will usually be taken of the bivalves themselves, although testing sewage and water samples can be used to investigate sewage treatment efficiency and as part of the shoreline survey/indicator survey process.

4.1.2.3 Pathogens

Recommendation

Primary monitoring for pathogens may be undertaken where the pathogens have been identified as potentially significant hazards in the Growing Area Risk Profile and there is a need to determine whether they occur, or occur in concentrations deemed to be unacceptable. It may also be undertaken to given background data against which to judge the outcome of targeted monitoring undertaken during events (see Section 6.2).

Monitoring for pathogens should generally be undertaken in the bivalve mollusc itself. Where only parts of the bivalve are to be consumed, analysis may be undertaken for the pathogen only in the part(s) that are eaten. Care needs to be taken to ensure that this is based on consumption patterns that apply to all potential consumers of the product.

Other considerations

Further to the advice given in CAC (2010a), where the risk profile has identified that *V. vulnificus* and/or *V. parahaemolyticus* may be hazards relevant to the growing area, monitoring of bivalve molluscs at harvest for the levels of total *V. vulnificus* and total and pathogenic *V. parahaemolyticus* should be conducted to determine the regional and seasonal variation.

If monitoring for other specific pathogens is to be undertaken in response to expected or unexpected events (see Section 6), then it will be appropriate to consider undertaking a baseline survey of the occurrence and concentration of the hazard(s) in the growing area so that the results obtained following an event can be assessed against the baseline.

Primary detection or quantification of pathogens by molecular (such as PCR) or immunological methods may give positive reactions with inactivated microorganisms. For some microorganisms, such as norovirus, it is not presently possible to confirm the results by viability assays. However, methods are under development which seek to determine whether detected virus is viable. For some other pathogens, the molecular or immunological methods may be more sensitive and/or specific than conventional culture methods and so use of the latter methods may not necessarily enable confirmation of results obtained by non-cultural methods.

Explanation

Monitoring of pathogens in the bivalve mollusc is usually appropriate due to the fact that the concentration of the pathogen that is measured can then be directly related to the risk to health arising from consumption. Differences in uptake of contaminants by different bivalve species means that either all harvested species will need to be monitored separately, or one or more indicator species will need to be identified that are protective in terms of public health in terms of uptake kinetics (i.e.

contamination events are reflected at least as quickly as the other species), depuration kinetics (i.e. after contamination events the hazard is present at least as long as in other species) and maximum concentrations.

4.1.3 SAMPLING SITE SELECTION

Recommendation

In general, for faecal indicator monitoring, one or more sampling sites should be identified within the growing area that reflect the worst-case situation with respect to the sources of contamination that have been identified during the growing area assessment. The site(s) may reflect a spatial integration of expected levels of contamination from multiple sources rather than being targeted at individual sources. In general, the larger and more complex an area, in terms of the number and types of sources (where relevant to the indicator/hazard to be monitored), the more sampling sites should be identified in order to reflect the magnitude and variability within the area. It is generally the case that more stations will be identified for water monitoring than for bivalve monitoring, as with water there is not the advantage of integration of contamination seen with bivalves.

For water monitoring, boundaries of the growing area, the identified impact points of sources, including discharges and watercourses, should be targeted for location of sampling sites for indicators and pathogens related to faecal pollution. For bivalve monitoring, sampling sites should be located on the bivalve resource at locations where the integrated risk of contamination from identified sources is deemed likely to be highest. For pathogens that are not related to faecal sources, sampling locations should either be targeted at locations where known factors may relate to an increased risk of occurrence (e.g. temperature and depth for some Vibrio spp.) or should be sited at locations across the bivalve resource.

For bivalves that are being cultivated through a depth (e.g. on lines or bouchots) the location should include specification of a depth range based on an expectation of the location of the contamination within the water column (e.g. if stratification occurs). For water, samples are generally taken immediately subsurface as any stratification will tend to lead to higher results at that location (freshwater inputs from sewage discharges and watercourses will tend to be near the surface). However, in locations where mixing is not expected to occur, bivalve resource located at depth may not be exposed to contamination constrained at the surface and sampling of water at the depth of the resource may be more appropriate. In addition, where information indicates that higher levels of contamination may occur at other depths, and this is relevant to the bivalve resource, sampling should be targeted accordingly. Sedimentation of solids associated with raw or primary-treated sewage and re-suspension of contaminated sediment are both processes that may result in higher levels of contamination at depth.

Other considerations

Where the expected worst-case location(s) lies away from the current bivalve resource, bagged or caged bivalves may be placed at this location for the purposes of sampling. However, this approach is not suitable for all species of bivalves (e.g. many species of clams will not survive for extended periods of time in such conditions). The alternative approach is to constrain the area to be classified to ensure that one or more sampling points on the present resource properly reflect the worst-case impact of the identified sources. The number of points depends on size and complexity (sources, tidal effects) of the area and the expected (or known) variability in presence, or concentration, across the area.

When identifying sampling sites, it is also necessary to identify the allowed distance that samples may be taken from the stipulated location and still be deemed to be valid. This is necessary in order to ensure that a sample represents the intended sampling site and to yield comparable time series data. Allowance of such a tolerance around the identified location is also important with bivalve sampling as resource may not always be available at the specified point on each occasion. This is especially a problem with wild fisheries where the location and density of resource may vary with time. If sufficient animals cannot be obtained within the specified tolerance of a bivalve sampling site on more than one occasion, designation of an alternative site will need to be considered. This should be done with reference to the Growing Area Assessment so that it is located appropriately. The new sampling site should be given a separate identifier so that the data associated with it can be separately identified during subsequent Growing Area Reviews.

Explanation

The selection of sampling sites will be affected by the contaminant being monitored and the matrix. Growing areas in well-mixed water bodies will generally require fewer sampling sites then those in bodies that are not well-mixed.

More sampling sites will usually be identified for primary monitoring than will be used for ongoing monitoring as the results of primary monitoring will enable redundant sites (those yielding no additional useful data) to be identified and removed from the programme.

4.1.4   SAMPLING STRATEGY

Recommendation

Two alternative approaches to sampling may be applied.

> Random sampling. Ideally, this should be undertaken according to a predefined schedule to be random with respect to any likely influencing environmental factors e.g. tidal state, rainfall, wind, etc., so as to avoid introducing any bias to the results. Where conditions, such as extreme adverse weather, prevent a planned sampling from being undertaken, an additional sampling occasion should be

identified on the same basis as the main programme. In some areas, practical constraints (access at certain tidal or weather states, daylight availability, etc.) mean that a truly random approach cannot be applied. In that case, the schedule should be defined to be random with respect to all of the factors, or state of factors, for which this is possible. Alternatively:

> Adverse pollution condition sampling (worst-case approach). This is undertaken under conditions that have been identified as producing the highest levels of contamination. Adverse pollution condition sampling requires targeted sampling to evaluate the likely impact of a pollution event on a growing area. Sufficient sampling at each identified site is required to enable prediction of the events related to the condition and to assess the growing area when not affected by the event.

Other considerations

The U.S. NSSP identifies the use of systematic random monitoring for areas that are not affected by point sources, with adverse pollution condition monitoring being used for other areas.

Conditions that give rise to significantly higher levels of pollution may initially be identified during the growing area assessment and subsequently confirmed by the outcome of ongoing monitoring. However, the results from such monitoring will itself be affected by the basis taken to timing of sampling and this may bias any interpretation with respect to worst case conditions.

It may not always be possible to sample under worst case conditions due to access or other constraints. For example, sampling of bivalves from an intertidal area may not be possible at high tide and sampling by boat may not be possible at low tide. This can sometimes be overcome by placement of stock for sampling at an accessible point ensuring that such stock is otherwise representative of the harvestable resource). Alternatively, in some areas it may be possible to replace worst case timing of sampling by modification of sampling location (Lee, 2012).

Explanation

Random sampling may only intermittently reflect faecal contamination from some sources intermittently in areas where contamination in the water column is not well mixed or if the factors giving rise to the greatest level of contamination only occur occasionally. In such circumstances, worst-case sampling will tend to show higher results on a greater number of occasions.

Adverse pollution condition (worst case approach) sampling needs to be timed to determine if the conditions leading to the event can be predicted so that a management plan can be developed for a potential conditional area. An example includes sampling several days in a row after rainfall events to determine if an impact occurs and if so, how long it takes for the bivalve mollusc to cleanse naturally after the event. This will identify times when the area is significantly contaminated and then provide data to determine status of the area when not affected by the event (data showing the bivalve molluscs have cleansed).

4.1.5 SAMPLING FREQUENCY

4.1.5.1 Faecal indicator bacteria

Recommendation

Random sampling – For faecal indicator bacteria, at least two weekly sampling at each identified site is recommended for primary monitoring in order to provide sufficient data for classification at a first annual review.

Adverse pollution condition sampling – This requires targeted sampling to evaluate the likely impact of a pollution event on a growing area. Sufficient[23] sampling at each identified site is required to enable prediction of the events related to the condition and to assess the growing area when not affected by the event.

Other considerations

The appropriate sampling frequency will vary according to the matrix (and potentially species if the matrix is bivalve flesh), changes over time in pollution sources and local environmental factors and will need to reflect the variability of the indicator between years, seasons, and even over shorter timescales. The frequency should be set to reflect the known variability and, where a data set is to be assessed against specific statistical measures, also to provide sufficient data to analyse against such measures.

Explanation

For primary monitoring, which is undertaken over a shorter timescale than ongoing monitoring, obtaining sufficient data for a robust assessment against specific criteria means that a higher frequency is required than for ongoing monitoring.

Adverse pollution condition (worst case approach) sampling needs to be timed to determine if the conditions leading to the event can be predicted so that a management plan can be developed for a potential conditional area. For example, sampling several days in a row after rainfall events to determine if there is an impact, and, if so, how long it takes for the bivalve mollusc to cleanse naturally after the event. This will identify times when the area is significantly contaminated and then provide data to determine status of the area when not affected by the event (data showing the bivalve molluscs have cleansed).

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[23] The amount of sampling needed will depend on a number of factors, including the magnitude of the results under the adverse pollution condition compared those obtained under other conditions. The number of results under both the adverse pollution condition and other conditions should preferably be sufficient to demonstrate a statistically significant difference as well as a difference in any classification compliance assessment.

4.1.5.2 **Other indicators and pathogens**

Recommendation

An appropriate sampling frequency should be identified depending on the reason for monitoring (see Sections 4.1.2.2 and 4.1.2.3 and Annex 11), and the expected (or known) variability in presence or concentration over time.

Other considerations

As primary monitoring is undertaken for only a relatively short time, an initial sampling frequency of every two weeks (as for faecal indicator bacteria) may be used if there is no information on which to base an alternative frequency. This may be reviewed on an ongoing basis as data is obtained. However, any knowledge of potential seasonal (or other time-dependent) variations in presence or concentration needs to be taken into account before assuming that a short-term data set is potentially representative of longer-term conditions.

Explanation

The sampling frequency needs to reflect the occurrence or concentration with time of the alternative indicator or pathogen over time, including detection of peak events (and thus greatest risk). The frequency depends on the purpose of the monitoring, the characteristics of the area, and the sources or factors that affect the presence or concentration of the target micro-organism. Relatively frequent sampling during the primary monitoring phase will give good background information on which to base, or target, additional monitoring in relation to higher risk periods, or for the purposes of event management.

4.2   ONGOING MONITORING

The purpose of ongoing monitoring is to reflect the presence and/or occurrence of hazards (or indicators of these hazards) that may be relevant to the growing area, on either a continuing or an intermittent basis, in order to inform risk management procedures (classification, expected event management, unexpected event management). It confirms the continued status of an area with regard to any hazard and determines whether this status changes significantly. There is a need to balance the desire for a comprehensive programme needed to reflect any spatial and temporal variability in the hazard(s), against an approach that does not require disproportionate resources. Ultimately, the outcome is to protect public health and therefore any constraining of the number of sampling points and frequency of sampling should be directed towards reflecting the worst-case situation.

4.2.1 BASIS OF ONGOING MONITORING

Recommendation

The indicators/pathogens to be addressed in ongoing monitoring and the associated sampling plan(s) for the growing area (matrix(ces), number and location of sampling points, frequency of sampling) should be based on the first Growing Area Review (see Section 7), assessment of data from primary monitoring.

Other considerations

Most of the principles that apply to primary monitoring apply to ongoing monitoring. Thus, the following should be in place:

> training of samplers;

> sample collection protocol(s);

> sample transport protocol(s); and

> sampling plans.

Explanation

Ongoing monitoring is generally an extension of primary monitoring but with the target indicators/pathogens, and associated monitoring modified in the light of the results of primary monitoring and other information acquired for the first Growing Area Review. The content and targeting of the ongoing monitoring may be further revised as subsequent growing area reviews are undertaken.

4.2.2 INDICATORS/PATHOGENS TO BE MONITORED

Recommendation

Consider whether the range of indicators/hazards selected for primary monitoring are still appropriate.

Other considerations

The review may determine that ongoing monitoring for a specific indicator and/or pathogen is not necessary. For example, if the growing area assessment had identified a specific hazard as being potential, rather than actual, the outcome of primary monitoring will contribute to a decision as to whether that hazard is actually of concern with respect to the growing area.

It may also be the case that the review identifies that monitoring for additional hazards is required. For example, if the concentration of faecal indicator bacteria is elevated during specific periods, additional pathogen monitoring may be indicated during those periods.

The results from primary monitoring may indicate that there is no need for ongoing monitoring of one or more indicators and/or pathogens. This would normally be the case when the monitoring has shown that levels of the pathogen(s) are consistently deemed satisfactory. However, in this respect, the outcome of the growing area assessment needs to be taken into consideration as intermittent contamination events may not be apparent in the results of primary monitoring.

Evidence may be obtained through the Growing Area Review, including the results of primary monitoring, that ongoing monitoring for one or more indicators and/or pathogens should be targeted at certain times of the year or certain conditions. For example, if monitoring is undertaken for vibrios, this may be focused on times of the year when sea/air temperatures indicate a higher risk.

Where the level of one or more hazards is above levels deemed to be acceptable, and the area is subject to permanent closure, it may be decided to suspend monitoring for all hazards (see also Section 6.1).

Explanation

Except where dictated by existing legislating, the range of indicators/hazards selected for primary monitoring is based on the Risk Profile and initial Growing Area Assessment and address hazards that further evidence shows are not of concern. At the same time, additional evidence obtained during the review process may identify that additional indicators/hazards need to be included in the Growing Area Monitoring.

4.2.3 NUMBER AND LOCATION OF SAMPLING POINTS

Recommendation

Consider whether the number and location of sampling points needs to be modified.

Other considerations

If ongoing monitoring is necessary, fewer (or more) sampling points may be required, the location(s) may need to be amended and the sampling frequency or timing adjusted.

The results of primary monitoring may suggest, from the similarity of results, that some sampling points are yielding similar results. In this case, consideration can be given to omitting one or more sampling points deemed to be giving superfluous results. However, care needs to be taken as sampling points that are reflecting the impact of different sources of contamination may fortuitously give similar results. The following need to be taken into consideration:

> whether the Growing Area Assessment supports a contention that the sampling points may be reflecting the same sources;

> whether the timing of low and high results at the two (or more) sampling points coincides – this will be the case if the sampling points are reflecting the same sources; or

> where there is a difference in compliance, the point(s) that are kept in the monitoring programme should yield more high results than those that are removed.

The inclusion of more sampling points may be considered where there are significant differences in results between those that were used for primary monitoring or where the results from the existing points do not yield the results expected from the Growing Area Assessment (e.g. the plume from the outfall of a sewage discharge appears to be missing the present sampling point(s).

Explanation

The spatial variability of the results from the primary monitoring will provide an indication as to whether the number of sampling points can be reduced while still properly reflecting the impact from the relevant hazard(s). At the same time, if Growing Area Assessment (or Review) provided evidence of the probable presence of a hazard and this was not seen at the expected level in primary monitoring, it will be necessary to either add one or more sampling points or to move the location of one or more of those used for primary monitoring.

4.2.4 FREQUENCY OF SAMPLING FOR FAECAL INDICATOR BACTERIA

Recommendation

Consider whether the frequency of sampling for each indicator/hazard needs to be altered from that used for primary monitoring.

Other considerations

The level of a specific hazard (or indicator) together with the temporal variability seen in primary monitoring will provide an indication as to whether the frequency of sampling needs to be increased or can be reduced. For example, if all results for a specific hazard are markedly below the upper limit deemed to be acceptable, the sampling frequency may be reduced or, for some hazards, suspended. However, this needs to be considered with respect to the Growing Area Assessment (and Review) in order to determine whether the period of primary monitoring is likely to have covered all contamination scenarios (e.g. year-to-year or seasonal variation) and the likelihood of intermittent contamination events with respect to the hazard(s) in question.

The default frequency for sampling for faecal indicator bacteria is recommended to be monthly in order to provide sufficient data for classification assessment. However, this default frequency may be modified dependent on the nature of faecal sources identified during the Growing Area Assessment (and Review), including whether

intermittent contamination is of concern, the hydrography of the assessment area (in particular, whether the water column is well-mixed) and the variability of the primary monitoring results. If the results of primary monitoring have shown low variability, the frequency may be reduced to every two months. If the results are highly variable, the frequency should be increased to fortnightly.

Where harvesting is only undertaken for part of the year (seasonal harvest), sampling should be concentrated on the harvested period. However, sampling should begin one month before harvesting commences. The same number of results is still needed for assessment and so a higher rate of sampling will normally be required.

The frequency for adverse pollution condition (worst case approach) targeted sampling should generally follow the default frequency as above. But it is important to note that the predicted cleansing periods for adverse events need to be verified on an ongoing basis. Monitoring samples should be taken soon after the predetermined cleansing period has elapsed. Even during months where no adverse events, that impact on the area, occur it is appropriate to take samples that month during or soon after lesser events to reconfirm they do not impact. For example, if an area is only affected by 50mm rainfall in a 24-hour period and that rainfall (or greater) does not occur during the month, sampling could test a rainfall event <50mm in that month (e.g. a 20 mm event).

Explanation

In general, the frequency of sampling undertaken for primary monitoring is relatively high, so that sufficient data is obtained for consideration at the initial Growing Area Review. The review process will provide an objective basis for potential modification of the sampling frequency for each indicator and/or hazard.

4.2.5   FREQUENCY OF SAMPLING FOR OTHER INDICATORS AND PATHOGENS

Recommendation

The frequency of sampling for other indicators and pathogens should be determined on the basis of:

> the intent of the monitoring (see Annex 11);

> known factors that affect the occurrence or concentration of the indicator or pathogen (e.g. seasonality, wastewater treatment works malfunctions, heavy rainfall events); or

> known variability in the occurrence or concentration of the indicator or pathogen when the predisposing factors are present.

Other considerations

The intent of the monitoring may include a sampling plan for one or more specific pathogens in the absence of any indicator monitoring, reflecting the targeting of the

overall growing area programme, and depending on the outcome of the Growing Area Risk Profile. In such circumstances, the frequency and targeting of monitoring will differ from the circumstance where pathogen monitoring is undertaken in addition to relevant indicator monitoring.

The Growing Area Assessment may identify factors that affect the occurrence or concentration of pathogens in a growing area. Further information may be derived from any primary monitoring that may have been undertaken.

Explanation

The intent of monitoring for other indicators and pathogens is the primary driver in determining when and how frequently sampling is undertaken. That intent may be related to factors affecting the occurrence or concentration of pathogens (e.g. for enteric pathogens, events resulting in markedly increased sewage input to an area; seasonality effects for norovirus or pathogenic vibrios). If not, those other factors may be subsidiary but significant considerations in determining when to sample. Known variability in the occurrence or concentration of other indicators or pathogens will influence the frequency of sampling – if there is low variability then the frequency of sampling may be lower than if there is high variability – the intent being to capture the worst state that may affect public health.

Where conclusions on relevant factors or variability are not available from the Growing Area Assessment or primary monitoring, information may be obtained from other studies (in the assessment area, nearby geographical locations or elsewhere in the country) or even the scientific literature. Care needs to be taken when using information or data from locations outside the immediate area under consideration as the same factors or variability may not necessarily apply.

4.3   GENERAL CONSIDERATIONS

Several aspects of monitoring, such as recording of sampling plans, sampling, sample transport, laboratory analysis and data storage apply equally to primary and ongoing monitoring.

4.3.1   DOCUMENTATION OF SAMPLING PLANS

Recommendation

The sampling plan should be formally documented. The primary copy should be stored and maintained by the responsible authority but should be made available (either as controlled secondary copies or via electronic means) to those undertaking the sampling and laboratory testing, as well as to other relevant stakeholders.

Other considerations

The following are the minimum recommended items to be covered in the documented sampling plan(s):

> growing area identifier;

> sampling site identifier;

> matrix to be sampled;

> species (where bivalves are to be sampled);

> geographical location (grid reference and/or latitude/longitude);

> allowed maximum distance from identified sampling point;

> depth of sampling (if relevant);

> frequency of sampling;

> determinands to be tested;

> sampling body (where sampling is delegated by the responsible authority);

> authorized sampler(s): name(s) and reference number(s); and

> other relevant information.

Where multiple matrices are to be sampled in a growing area (e.g. both bivalve molluscs and water, or multiple species of bivalve molluscs), or more than one determinand (indicator, pathogen or other hazard) is to be tested for on the samples, it may be simpler to have separate sampling plans for each matrix and or determinand (or determinand type). This is especially the case where different criteria (such as location, depth and frequency) are specified for differing matrices or determinands.

Explanation

It is important to be able to trace the basis of sampling back to the recommendations from the growing area assessment. It is also important for representatives of the responsible authority, samplers and receiving laboratories to know what sampling and testing is intended to be undertaken, where and when. This is best achieved by formal documentation of the sampling plan(s) with distribution to relevant interested parties. In addition, documentation of the sampling plan(s) facilitates subsequent evaluation of compliance during the Growing Area Review or during an audit by the responsible authority or a third party.

4.3.2 GENERAL SAMPLING CONSIDERATIONS

Recommendation

Samplers should receive specific training relevant to the type of sample to be taken and the subsequent analysis to be performed. The sampling should be undertaken to specific protocols and the responsible authority should undertake periodic audits to

ensure that the samplers are adhering to the protocols. The protocols should cover: sampling methods, cleaning, packing, labelling, submission form and transport requirements.

Other considerations

The actual location and time of sampling should be recorded along with any specified environmental observations. The latter may include weather, tidal state, water temperature and salinity. Field observations at the time of sampling that are useful to record are: air temperature, notes of on-shore/off-shore human activities, presence of animals and birds, unusual condition or operation of known or potential sources of contamination (e.g. operation of a storm or emergency overflow on a sewage collection (sewerage) system). Samplers should be trained in the recording of such observations. Any measuring equipment that is used should be subject to periodic checks for accuracy.

Each sample should be given a unique identifier. This should be identified on the sample and any associated sample submission form (or equivalent electronic sample record system). The sample identification system should ensure traceability of the sample and associated information up to entry into the laboratory booking-in procedure.

An example sampling protocol is given in Annex 12.

Explanation

Sampling and sample transport protocols provide a standard approach to the conduct of these activities. Training of samplers should enhance compliance with the protocols. Traceability is necessary to ensure that the subsequent laboratory result relates to the sampling point to which it is assigned. Together, these provide confidence that the sample will be representative and correctly identified.

4.3.3 SAMPLING OF WATER

Recommendation

Sample size – For faecal indicator analysis, the sample size should generally be at least 300 ml (vary depending on what indicator is analysed). This will depend on the range of indicators to be analysed and the specific methods that are to be used. For bacterial pathogens, the sample size should be at least 1 Litre. For other analyses, the minimum sample size should be determined on a case-by-case basis with respect to any identified regulatory level or other objective by which the result will be assessed and the performance characteristics of the laboratory method to be applied.

Sample containers – Samples should be taken into sterile glass or plastic bottles, or purpose-made bags that have been shown not to adsorb, inactivate or otherwise alter the contaminant(s) for which sampling and analysis is being undertaken.

Sampling procedure – Care should be exercised at all times during the sampling procedures so as to avoid contamination. It is usually preferable to use a sampling pole with an appropriate clamp to take the size and shape of the sample container. This enables the sample to be taken away from disturbance by, and potential contamination from the sampler or boat. When the cap or seal is removed from the sample container, the inside of the cap should be protected. For sub-surface samples, the sample container should be inverted and immersed into the water to an appropriate depth, for example 300 mm. For static waters, the opening of the container should then be turned uppermost, allowing air to escape and the container to fill: it may be necessary to move the container gently from side to side can be encouraged by moving the container in a horizontal position with the neck of the container pointing away from sampling staff or boats. For flowing waters, the opening of the container should be positioned upstream to avoid contamination from sampler or boat. In general, a small air space should be left in the container after filling in order to allow proper mixing of the sample at the laboratory: this may not be appropriate for some chemical contaminants that may be subject to oxidation. The cap or seal should be replaced immediately after filling. This method generally avoids significant contamination with surface film. However, where this is a specific consideration, the cap or film should only be removed once the container has been immersed to the correct depth. For samples taken from a boat, it is preferable for the vessel to be pointed into the direction of any flow and the sample taken from the bow.

Other considerations

Sample sizes and other sample submission requirements should be agreed with the testing laboratory in order to ensure that samples are appropriate to requirements.

Additional information on the sampling of marine waters is given in Chapter 8 of Bartram and Reese (2000).

Explanation

Following appropriate procedures for the sampling of water ensures that the resulting samples are representative of the intended location and free from contamination from the sampler, sampling equipment and surface scum.

4.3.4 SAMPLING OF BIVALVE MOLLUSCS

4.3.4.1 Bivalve mollusc species

Recommendation

Either:

i.   separately monitor each species that is to be classified; or

ii. use an indicator species for the growing area

Other considerations

For the use of indicator species, parallel monitoring should show that the indicator species yields results at least as high as those of the other species it represents. This needs to be determined for each set of bivalve species and hazard combination. Care needs to be taken that the location of sampling of the indicator species is representative of the risk for the other bivalve species. For example, for faecal indicators, the sampling location for the indicator bivalve species should not be further from sewage or animal faecal sources than are the species it is to represent, unless a hydrographic assessment has shown that the selected sampling location is likely to be exposed to greater contamination.

Explanation

The key consideration is whether compliance, under whatever system is used, yields a classification for both the indicator species and any other represented by that species that is properly protective of public health.

4.3.4.2 Bivalve numbers

Recommendation

For faecal indicator bacteria, a sample for a single analysis should be comprised of at least 12 to 15 individual animals in order that at least 10 viable individuals can be tested on receipt at the laboratory. For small bivalve species, it may be necessary to collect a greater number of individual animals per sample in order to yield sufficient flesh for testing. For MSC or pathogens, the numbers should be as defined by the method, allowing for up to 20 percent of animals to become moribund prior to receipt at the laboratory.

Other considerations

For individual bivalve species, the required number of animals per sample should be determined in conjunction with the testing laboratory.

Explanation

The concentration of a hazard or indicator will vary markedly between individual animals and ensuring that at least 10 animals are tested reduces sample to sample variability. Use of a smaller number of animals should be supported by a demonstration that the variability of results obtained for the hazard or indicator is not significantly greater than that obtained with the recommended number. It is preferable to collect more animals than required for the test in order that the minimum specified number for analysis is still satisfied if a proportion become moribund or die during transit.

4.3.4.3 Sampling procedure

Recommendation

Where possible, the method normally used for harvest in the specific growing area should be used to obtain monitoring samples. The temperature of the sample (either the test portion for bivalve molluscs or the surrounding seawater) should be recorded. After sampling, any detritus should be removed from the shells by rinsing, if necessary in combination with brushing, preferably using clean water[24]. An acceptable alternative is seawater from the vicinity of sampling. The bivalves should not be immersed during the cleaning procedure in order to avoid uptake of dirty water. The animals should then be drained well and then be placed in a food grade polythene bag. The bag should be securely closed and then labelled (if it is not pre-labelled with the appropriate identifier).

Other considerations

It may not be possible to obtain samples by the method usually used for harvesting if that method uses equipment that is not available to the sampler or if the location of sampling (e.g. a specific depth range on mussel lines) cannot be sampled using that method. In the latter case, it may be acceptable to obtain the sample during normal commercial harvesting with the sample collection under official supervision in order to ensure that the requirements of the sampling plan and the sample collection and transport protocols are satisfied.

Explanation

The sampling procedure will vary according to the bivalve species and nature of the fishery. Use of the normal harvesting method to take the sample will usually ensure that samples can be obtained and that any additional contamination caused by the harvesting method (e.g. uptake of disturbed sediment) will be reflected in the samples. Proper cleaning of samples, followed by draining, prior to being placed in bags is necessary to avoid the possibility of uptake of contaminated water by the bivalves during transport. The use of properly labelled, sealed bags, in conjunction with traceability within the laboratory, ensures that an audit trail can be maintained from sampling to analytical result.

4.3.5 SAMPLE SUBMISSION FORMS

Recommendation

A sample submission form should be completed for each sample. As a minimum, this should contain the following:

> time and date of sampling;

> unique identification number (as given on the sample container);

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[24] Clean water is water from any source where harmful microbiological contamination, substances and/or toxic plankton are not present in such quantities that may affect the safety of fish, shellfish and their products intended for human consumption.

> sample type;

> growing area identifier;

> sampling point identifier;

> sampling location (determined by GPS);

> sampler; and

> analysis type, together with:

> the results of any environmental testing (sample temperature, salinity, etc) specified by the sampling protocol; and

> additional observations and comment: e.g. specific observations on abnormal weather conditions, presence of unexpected pollution sources or activity in the vicinity, sample state.

Other considerations

An example sample submission form is given in EURL (2017).

Explanation

Provision of full details relating to the sample and sampling conditions gives information to the laboratory that may be relevant to determining analytical requirements and also to the subsequent assessment of the analytical result by the responsible authority.

4.3.6   SAMPLE TRANSPORT

Recommendation

Water samples should be placed in light-proof boxes for transport. The temperature of microbiological samples during transport should reach between 0°C and 10°C within 4 hours of sample packing, and then be maintained within this range until receipt at the laboratory. If cool packs are used samples should not come into direct contact with their surfaces. Samples should not be frozen. Microbiological analyses should be commenced within 24 hours of sampling. Sample containers should be closed and separated to prevent cross contamination among them.

Other considerations

If initiation of a microbiological analysis cannot be undertaken within 24 hours of sample collection, or if sample transport or storage temperatures are outside the recommended range, verification studies should be undertaken to support the use of those conditions. For other types of contaminant, verification studies should be undertaken if the contaminant is not known to be stable under normal transport time/temperature conditions.

An example sample transport protocol is given in Annex 13.

Explanation

The concentration of microbes in a sample can change markedly during transport over time, especially if temperature conditions are not kept within a defined range. The change may be an increase, or a decrease, depending on the microbe and the conditions (including sample type). Other types of contaminant may or may not be stable over a range of conditions and knowledge of the stability with respect to sample type, temperature and time is necessary in defining appropriate sample transport criteria.

4.3.7 LABORATORIES

Recommendation

Laboratories undertaking testing of samples in support of a sanitary programme should be accredited to EN ISO/IEC 17025 for the specific method(s) to be used under the programme, unless an equivalent alternative accreditation is specified under regulations or to meet trade requirements. Laboratory staff undertaking analyses should be trained and assessed as competent for each method×matrix combination. Laboratories should undertake, for each matrix and analysis performed, appropriate internal quality assurance procedures, including the processing of internal quality controls and participation in a relevant proficiency testing scheme(s).

Explanation

Accreditation, staff training, quality assurance (including use of relevant internal quality controls) and participation in relevant proficiency testing schemes are essential components in providing assurance to both the laboratory and stakeholders that the results produced by the laboratory are valid.

4.3.8 MICROBIOLOGICAL METHODS

Recommendation

Recognized, and preferably properly validated, methods should be used within a sanitation programme in order that results are accurate, and reliable. The performance characteristics of such methods have generally been established. However, other methods that yield equivalent, or better, performance characteristics than the established, recognized methods may be used. The use of specific methods may be dictated by local regulations or trade requirements.

Standard microbiological methods deemed satisfactory for use in a bivalve mollusc sanitation programme are as given in Table 4.1.

TABLE 4.1 **RECOGNIZED MICROBIOLOGICAL METHODS**

MATRIX	TARGET ORGANISM	METHOD
Bivalve molluscs	Sample preparation for all bacteriological methods	ISO 6887-3
	Preparation of dilutions of homogenized samples for all bacteriological methods	ISO 6887-1
	E. coli	ISO 16649-3 (5 tube format)
	MSC	EURL generic protocol (EURL 2007) FDA MSC Method
	Salmonella spp. (detection)	ISO 6579-1
	Salmonella spp. (quantification)	ISO 6579-3
	Pathogenic vibrios	See FAO/WHO (2016)
	Hepatitis A virus and norovirus (quantification)	ISO/TS 15216-1
	Hepatitis A virus and norovirus (qualitative detection)	ISO/TS 15216-2
Water	Faecal coliforms and presumptive *E. coli* by membrane filtration	ISO 9308-1
	Faecal coliforms and presumptive *E. coli* by Most Probable Number (MPN)	ISO 9308-2
	MSC	ISO 10705-1
	Standard Methods for the Examination of Water and Wastewater (APHA, 1985)	APHA

Other considerations

International methods for the pathogenic ***Vibrio*** spp. are currently under review. Guidance on the selection of methods for pathogenic vibrios has been published by FAO and WHO (FAO/WHO, 2016).

It may be difficult to demonstrate equivalence of performance for new methods based on totally different analytical approaches (e.g. a molecular method versus an established culture based method). In such cases, it needs to be determined by other means whether the alternative method is fit for purpose.

Explanation

Where appropriate ISO methods are available, these are usually referenced in Codex codes of practice, standards and guidelines. A number of countries apply systems based on the U.S. NSSP, using methods stipulated in those guidelines (FDA, 2015).

4.3.9 OTHER METHODS

Recommendation

Where available, internationally recognized methods should be used for the detection or enumeration of other hazards, including other pathogens. The responsible

authorities should ensure that the performance characteristics of any method are appropriate for the use to which it is to be applied. This includes determining whether a detection or quantification method is applicable for the hazard and purpose under consideration.

Alternative methods, including rapid and/or molecular methods, should be validated against the above methods if they are to be used as part of a sanitation programme. However, such alternative methods may be used without validation in investigations or research associated with a sanitation programme: in such situations, the results of the primary or validated alternative methods should be used for classification and enforcement purposes.

Other considerations

Internationally recognized methods for the detection or enumeration of other pathogens, may not have been evaluated for testing bivalve molluscs. If not, then the suitability of the method should be determined using the species of interest within the sanitation programme.

Explanation

Any method where the results are to be used as the basis for risk management decisions should conform to accepted performance criteria. This includes methods for hazards or indicators that are not specified in regulations.

4.3.10 REPORTING OF LABORATORY RESULTS

Recommendation

Laboratory results should be reported to a timescale and format agreed between the responsible authority and the laboratory. Additional requirements may be dictated by the accreditation body. The agreed timing between sample receipt and reporting needs to accommodate the full period needed to conduct the analysis according to the method in question, including quality assurance and validation checks. However, consideration also needs to be given to any short-term actions that may be taken on receipt of atypical results: therefore, reporting should take place as soon as practically possible after the laboratory procedures have been completed.

Other considerations

Where a Laboratory Information Management System (LIMS) is used (see below), an automated reporting procedure may be instituted. However, such automated reports should not be sent prior to completion of the laboratory procedures, including validation and authorization. Unless otherwise agreed between the parties, the responsible authority should communicate results to stakeholders as this allows for the inclusion of other relevant information and interpretation.

Laboratory results should be accompanied by an estimate of the uncertainty of measurement for the specific method used in the reporting laboratory.

Explanation

The reporting stage for laboratory results is a final step in the analytical process and is an essential component of the audit trail from sampling to result. Prompt reporting of laboratory results is essential where these may be used as the basis for risk management actions or for triggering growing area investigations.

4.3.11 DATA STORAGE

Recommendation

Relevant information from a monitoring programme should be stored in a suitable form such that it can be readily retrieved when needed. It should also be possible to cross-relate aspects of this information.

Other considerations

It is also necessary to ensure that backup records are kept in case the primary records are lost or corrupted. While such requirements may be met for a very limited programme by a paper system, it is more effective for most programmes to use computerized storage. Many of the records for a monitoring programme will have a geographical component (e.g. sampling locations) and it is therefore beneficial to either have a storage system which is part of a Geographical Information System or which can be linked to one (e.g. via export of relevant data).

Where possible, it is useful to incorporate other data from the sanitation programme in the GIS (e.g. growing area boundaries, bivalve resource locations, sources of pollution) so that monitoring results or other relevant data (such as the location of a sewage spill event) can be viewed in the broader growing area context in map form. Entry of data into computer storage systems may be done manually from hard copy records (e.g. sample submission forms) or the original data may be entered directly into a computerized system (e.g. a waterproof tablet) and then transferred to the main data storage system.

Laboratory-specific data and results may be maintained within a LIMS. Such systems enable the storage of sample details, data from key stages of laboratory methods, quality control results and sample results in one system in a way that the details relevant to a single sample, or batch of samples, can be readily retrieved. In addition, an audit trail is maintained in relation to both the laboratory activities relating to a sample and any changes to the records. There are several off-the-shelf packages available and one of these may provide a ready-made solution to the data storage needs of a laboratory. However, the laboratory should determine that a specific package is able to satisfy its own needs, other requirements of the programme, and that it will be acceptable to the relevant accreditation body. Automated reports produced by a LIMS after result validation may be directly assimilated by a computer-based monitoring programme database. In order to ensure that the correct results are connected with the correct samples and sampling points, it is important

that it is possible to track through from sampling through to incorporation in the database: this is most easily achieved by the use of single specific sample identifiers.

It is important that any system, manual or computerized, contains validation checks for as many of the entries as possible. This may be achieved by restricting entries to a number of options via a drop-down list, by restricting entry format to a specific type (e.g. date, text or integer) or by having in-built checks that values fall within a sensible range. In the latter case, it should be possible override the system to enter true values outside that range but an associated comment should be required.

Any record system, computerized or manual, should have an audit trail for changes to data. Computerised systems should automatically record who made a change and the associated date and time and should require a comment as to why the change was made. The same information should be recorded for changes made on paper records – in such cases, deletion of the original data should be made in such a way that the original entry can be discerned and all changes should be made in permanent ink.

All data entered into the storage system should be retrievable by appropriate personnel. Standard queries and reports should be provided in order to retrieve the most commonly required data easily and in a consistent format. It is useful for data with a geographical component (i.e. a location) to be displayed on a map as this aids interpretation within the broader programme context.

Explanation

Operation of a sanitation programme can generate large amounts of information and data. This needs to be stored in such a way that the data is readily retrievable and also enables comparisons among different but related elements of the programme, e.g. sampling plans and actual sampling locations; and sample results from different sampling points. Validation at data entry provides confidence in outputs retrieved from the system.

CHAPTER 5
CLASSIFICATION

Classification constitutes a third decision point, based on the outcome of the growing area assessment and primary monitoring.

5.1 INITIAL CONSIDERATION – PURPOSE OF CLASSIFICATION

Classification provides a broad risk categorization for an area so that common risk management procedures and processing requirements can be applied that are readily recognized by regulators, enforcement staff, industry and purchasers. The procedures for assessing risk associated with hazards that lead to that categorization comprise the Growing Area Risk Profile, Growing Area Assessment and results from Primary Monitoring. For the purposes of classification, monitoring results provide an estimate of risk into the near- to mid-term future based on past performance. Over and above this, individual results may contribute to determination of conditional classifications (see Section 5.6) and may also contribute to other risk management actions (see Section 6).

5.2 COMPONENTS OF THE CLASSIFICATION PROCESS

Classification should involve the following elements.

5.2.1 DEFINITION OF THE GROWING AREA BOUNDARIES

Recommendation

The boundaries of the growing area to be covered by the classification should be explicitly defined using geographical co-ordinates. The growing area should be given a unique identifier (number and/or name) so that it can be unequivocally cross-related in different documents (e.g. sampling plans, classification listings, closure notices). The extent of the growing area should be based on the outcome of the Growing Area Assessment.

Other considerations

Where the concentration of a relevant hazard is deemed to vary markedly across an area (determined on the basis of the growing area assessment and/or monitoring), and harvest of the bivalve mollusc resource in separate parts can be managed and enforced separately, consideration should be given to designating separate growing areas for ongoing classification, monitoring and event management. In such cases, the boundaries of each growing area should be separately defined, a unique identifier given to each growing area and a sampling plan defined for each growing area for each hazard under consideration.

Explanation

It is necessary for the location and extent of the growing area to be evident to all stakeholders, including the industry and control authorities in order to assist compliance and enforcement.

An example map showing the relationship between the assessment area, the classified growing area and the bivalve resource is shown in Figure 5.1. This is diagrammatic and the extent of the growing area will depend on the conclusions of the Growing Area Assessment, based on the analysis of hazards (including sources, where relevant) and the hydrography of the area.

5.2.2   DEFINITION OF AREAS THAT ARE UNSUITABLE FOR HARVEST

Recommendation

Any parts of the assessment area that are determined to be unsuitable for harvest for trade for human consumption should be designated separately to the area(s) to be harvested. The location and extent should be explicitly identified (preferably by means of geographical co-ordinates) and the area may be given a unique identifier in order to assist surveillance and enforcement.

Other considerations

Determination of unsuitability for harvest may be made on the basis of any relevant hazard: microbiological contamination, chemical contaminant(s) above acceptable levels, long-term presence of biotoxins above acceptable levels.

Explanation

There will be circumstances where a part, or parts, of an intended harvest area may be deemed as unsuitable for any harvest for eventual consumption: this will be where any form of post-harvest processing will not result in a product acceptable for human consumption.

FIGURE 5.1. **EXAMPLE MAP SHOWING THE RELATIONSHIP BETWEEN ASSESSMENT AREA, GROWING AREA AND BIVALVE RESOURCE**
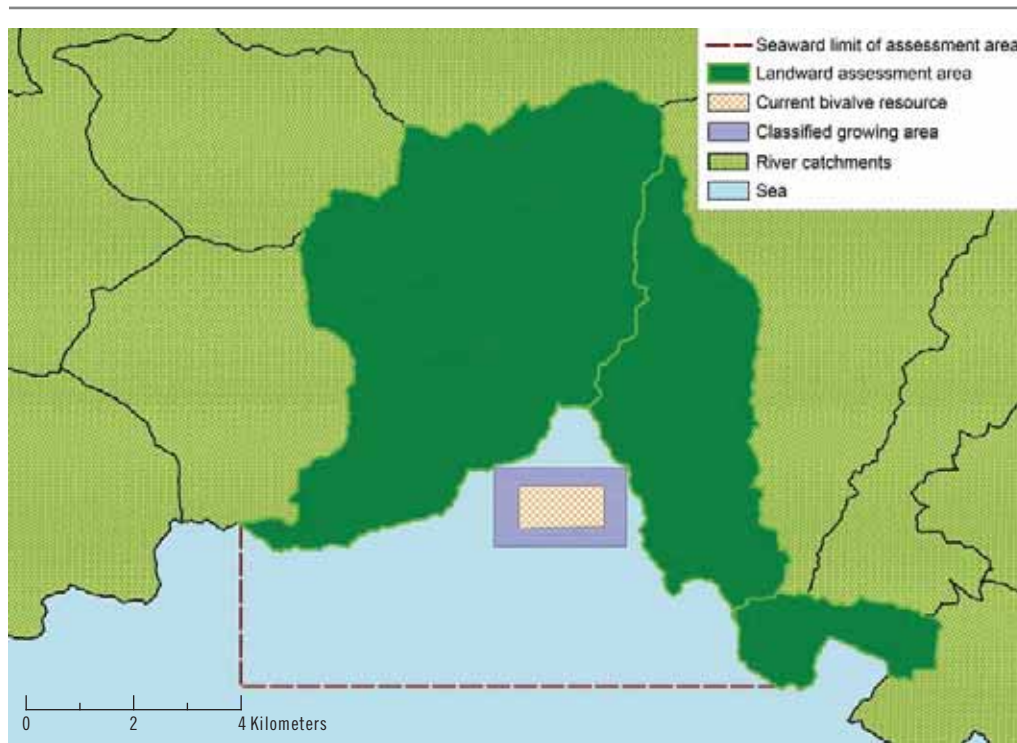
Contains public sector information supplied by the Environment Agency (England) and licensed under the U.K. Open Government Licence v3.0.

5.2.3   DETERMINING THE CLASSIFICATION LEVEL FOR THE GROWING AREA

Recommendation

Determine the classification level of the area on the basis of the Growing Area Assessment and the outcome of Primary Monitoring.

Other considerations

In order to define the classification level, it is necessary to define whether the requirements for classification are stipulated by any existing national or multinational (e.g. EU) regulation or whether there are any other requirements that need to be met for trade purposes. It may be the case that both situations apply to either some or all growing areas. Sometimes, the trade requirements may apply to a subset of growing areas that are approved for export. Where more than one set of requirements exist, it is necessary to identify the growing areas to which they apply and to ensure that the programme complies with all. If there are no pre-existing requirements, the public health objectives of classification should be defined: there is then the possibility

of using criteria from an existing programme applied elsewhere, if these properly satisfy the objectives, or developing other criteria that will meet those public health objectives. Further information in given in Sections 5.3 and 5.4.

Explanation

The classification level of a growing area determines the level of post-harvest treatment and thus to ensure that microbiological hazards of faecal origin (human or animal) are at a level deemed to be acceptable after such treatment.

5.2.4   DEFINITION OF CONDITIONAL CLASSIFICATION CRITERIA

Recommendation

Define any criteria which the growing area must meet to be deemed to conform to the classification at a specific level (e.g. classification dependent upon environment factors such as season, rainfall, river flow or salinity).

Other considerations

Areas may be classified at one level and be closed when the criteria for that level are not met or may be classified at two different levels.

The growing area should fully conform to the criteria for the classification level during the period when it has open status and to the criteria for each level where a two-level classification applies. In additional, where the criteria are specified on the basis of indicators (e.g. faecal indicator bacteria), the timing of the open status or better classification level should also allow clearance of the relevant hazards to acceptable levels.

Explanation

Some growing areas are subject to additional contamination under defined conditions (e.g. season, rainfall, river flows). The use of conditional classifications allows a growing area to be given a better category of classification when such conditions do not apply (to the benefit of the bivalve harvesters) and a worse category when they do. The alternative would be for the area to be classified at the worse category all of the time.

5.3   TYPE OF CLASSIFICATION

The following classification categories are, in general, based on the general risk from enteric pathogens as reflected by faecal indicator monitoring results. However, the risk from other hazards should also be considered where appropriate. Conditionally managed areas may be assigned two different classifications, that applying at a specific time depending on the criteria that are applied.

Category I - Fit for direct human consumption

All of the bivalves in each batch of product harvested from such an area should be fit for consumption without any subsequent processing (ignoring the usual activity of washing prior to packing). Therefore, all potential hazards identified in the risk profile should either be absent or, if present, be at levels deemed to be acceptable.

Category II - Need for depuration or short-term relay

Bivalves subject to relatively low levels of faecal contamination have been traditionally subjected to depuration in order to reduce the risk from enteric pathogens. Short-term relay in the natural environment has been undertaken to achieve the same objective. It is recognized that such processes do not adequately reduce the risk from enteric viruses or vibrios. Therefore, all potential hazards identified in the risk profile should either be absent or, if present, be at levels deemed to be acceptable. Additional risk management measures may be necessary in order to ensure that this is the case.

Category IIIa - Need for long-term relay

Long-term relaying (e.g. for two months) may be used for the reduction of contamination to an acceptable level for human consumption. In general, such relaying has been used to reduce the risk of viral illness associated with bivalves grown in areas exposed to moderate levels of faecal contamination. Consistent reduction of chemical contaminants and biotoxins has not been demonstrated over such periods. Longer-term (e.g. at least six months) on-growing of seed bivalve molluscs in cleaner areas has been used to reduce concentrations of some chemical contaminants to acceptable levels and also for the reduction of enteric viruses in seed bivalves taken from areas exposed to high levels of faecal contamination.

Category IIIb - Need for post-harvest treatment (high pressure, cooking, canning, freezing)

With respect to a sanitation programme, post-harvesting treatment is either used as an alternative for depuration or relay or it is used as an additional process to inactivate pathogens that are not satisfactorily removed by those other procedures. High-pressure treatment, cooking and canning under appropriate controlled conditions will inactivate enteric viruses and vibrios. Freezing will reduce the concentration of pathogenic vibrios. The process needs to have been proven to effectively reduce the contaminant of concern to acceptable levels.

> Therefore, high pressure treatment, cooking and canning may be applied to bivalves harvested from Category II areas (EU class B; US restricted).

> High pressure treatment, cooking and canning may be applied to bivalves harvested from any category of area during periods when the risk associated with pathogenic vibrios is elevated.

> The processes may be applied to bivalves harvested from Category I areas with no elevated vibrio risk, for purely commercial purposes.

> Efficacy data is needed for the process in relation to the hazards of concern in order to determine the appropriate options post-harvest.

Category IV - Not fit for human consumption in the form generally consumed (no mitigation available to address the relevant contaminants)

> Growing areas that have not been subject to a risk profile, growing area assessment and primary monitoring should, by default, be considered unsuitable for gathering for human consumption.

> Growing areas should be designated as prohibited for gathering for human consumption if they are determined to be subject to faecal contamination to an unacceptable extent, or one or more specific hazards is present at levels that may be harmful to human health. This may be ascertained on the basis of the risk profile, the growing area assessment, primary or ongoing monitoring, or any combination of these.

> Growing areas should also be designated as prohibited for gathering for human consumption if the growing area assessment has identified that the area is likely to be subject intermittently to one or more specific hazards at levels that may be harmful to human health and the risk from that(those) hazard(s) cannot be properly managed.

> The responsible authority may allow gathering from buffer zones (areas around sewage discharges) for the purpose of removal of microbiological hazards as long as the bivalve molluscs are not exposed to unacceptable levels of other contaminants that will not be reduced to acceptable levels during such relay. Where there is no explicit evidence to demonstrate that this will occur, the responsible authority should only allow the removal of seed bivalves that will be ongrown in a classified area for at least six months.

5.4   CLASSIFICATION CRITERIA

Recommendation

The following items should be taken into account in determining the initial classification for an area:

> Risk Profile – with respect to the hazards relevant to the growing area;

> Growing Area Assessment – with respect to the sources of those hazards (where appropriate) and the impact of those sources within the growing area; and

> Results from primary monitoring – presence, or concentration, of indicators or hazards in the Growing Area.

Monitoring can seldom be comprehensive and therefore the results from monitoring only comprise one aspect of the classification of a Growing Area.

Category I

The results of faecal indicator monitoring should comply with the requirements for such an area, and bivalve molluscs harvested from such areas should not contain any hazards at levels deemed to be a risk to human health.

With respect to microbiological contamination, the Codex standard for live and raw bivalve molluscs specifies the following:

"Growing Area monitoring programmes, 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.

In analysis involving five (5) 100 g 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 one (1) of five (5) samples may contain between 230 and 700 *E. coli*, or equivalent as decided by the responsible authority having jurisdiction

Micro-organism = *Escherichia coli* n=5; c=1 m=230; M=700.

where 'n'= the number of sample units, 'c'= the number of sample units that may exceed the limit 'm', and 'M' is the limit which no sample unit may exceed." (CAC, 2014).

It is widely recognized that, while levels of *E. coli* are useful indicators of recent faecal contamination, they do necessarily relate to the presence and concentration of pathogenic micro-organisms of enteric origin (especially viruses and protozoa) and also do not relate to the presence and concentration of naturally occurring pathogenic marine vibrios. Compliance with the Codex standard for *E. coli* therefore does not necessarily ensure that live and raw bivalve molluscs are safe for human consumption and consideration needs to be given to the outcome of the risk profile and growing area assessment in determining whether other criteria should be satisfied for product intended for direct human consumption.

Countries using a faecal indicator system based on water monitoring usually apply the criteria specified in the United States National Shellfish Sanitation Programme (NSSP). The criteria specified for growing areas where product is to be marketed directly for human consumption (areas classified as approved) are given in FDA (2015). Other criteria may be used to assess the results of a water-based monitoring programme as long as the bivalves harvested from areas classified as Category I would conform to the requirements given in the Codex Standard. This does not mean that bivalves need to be monitored on a regular basis to demonstrate this. However, the criteria should be validated against the requirements in the Codex Standard and relevant verification should be undertaken. The latter may be accomplished by reviewing the data obtained at the packing establishment rather than necessarily undertaken verification sampling from the growing area.

With respect to other contaminants in live and raw bivalve molluscs, the Codex Standard identifies that:

"The products covered by this Standard shall comply with the Maximum Levels of the General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995[25]) and the maximum residue limits for pesticides and/or veterinary drugs established by the Codex Alimentarius Commission." (CAC, 2014).

Category II

The results of faecal indicator monitoring should comply with the requirements for such an area, and bivalve molluscs harvested from such areas should not contain any hazards at levels that will not be deemed to be a risk to human health after depuration or short-term relay.

The following approach is recommended when there are no existing regulatory requirements:

1. Identify from the risk profile those hazards that may need to be addressed by depuration or short-term relay (essentially bacterial enteric pathogens associated with faecal contamination).

2. Determine the depuration kinetics of the process to be used in relation to the hazard(s)(see FAO, 2008).

3. Therefore, determine the maximum concentration of the hazard(s) that will consistently yield product complying with the requirements for Category I at the end of the process.

4. Where there is a direct relationship between the concentration of a faecal indicator and the likely presence, or unacceptable concentration, of the hazard in bivalves, determine the 90th percentile faecal indicator concentration that relates to the maximum concentration of the hazard determined in iii. This faecal indicator concentration may be determined in either water or bivalve flesh, as required for the programme being implemented.

5. Review the results obtained from samples taken post-depuration/relay to confirm that the criteria for Category I are met by the depurated/relayed product.

Alternatively, in the absence of a criterion or criteria based on this approach, one of the following may be used:

> the U.S. NSSP criteria for restricted areas (based on faecal coliforms in water) (see Table 5.1)

> or the EU criteria for class B (using *E. coli* in bivalve flesh and intravalvular fluid) (see Table 5.2)

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[25] The levels are reviewed and, where necessary, revised periodically but the date of the original standard is maintained,

The use of these criteria may not provide adequate assurance that pathogens of enteric origin are at acceptable concentrations after post-harvest treatment and, unless validation has been undertaken to ensure that this is the case, additional risk management should be considered.

Over the review period (at least 24 results), the maximum concentration of the hazard(s) or indicator(s) should comply with the requirements for Category II. If hazards have been identified in the risk profile that are not reduced to an acceptable level by depuration/short-term relay, these should be addressed by separate monitoring and management procedures (either closures or additional processing requirements – see Category IIIb).

TABLE 5.1 **CLASSIFICATION CRITERIA UNDER THE U.S. NSSP**

| CLASSIFICATION | FAECAL COLIFORMS PER 100 ml WATER | | TREATMENT REQUIRED |
	GEOMETRIC MEAN[1]	90% COMPLIANCE[2]	
Approved Areas[3]	≤14	≤43	None
Restricted Areas[4]	≤88	≤260	Depuration[5] or relaying in an approved area
Prohibited Areas	No sanitary survey, or conditions not met for approved or restricted areas[6]		Harvesting not permitted

1. Or median;
2. Values for 5-tube decimal dilution test – a different 90 percent compliance is given for the 3-tube MPN and mTEC membrane filtration tests;
3. Determination of approved area status must be based on a minimum of 15 samples from each monitoring station.
4. Conditionally restricted areas may be declared where these are subject to predictable contamination events: such areas are closed for harvesting during contamination events and for a period afterwards to permit natural cleansing.
5. Depuration and purification are alternative terms applied to the process by which bivalve molluscs are held in tanks of clean seawater under conditions that maximize natural filtering activity, and which results in expulsion of intestinal contents, enhancing separation of the expelled contaminants from the bivalves, and preventing their recontamination (FAO, 2008).
6. Considerations other than the concentration of contaminants may be used to declare an area prohibited.

TABLE 5.2 **EU CLASSIFICATION CRITERIA**

CLASS[1]	MICROBIOLOGICAL STANDARD[2]	POST-HARVEST TREATMENT REQUIRED
A	Samples of live bivalve molluscs from these areas must not exceed, in 80% of samples collected during the review period, 230 *E. coli* per 100 g of flesh and intravalvular liquid. The remaining 20% of samples must not exceed 700 *E. coli* per 100 g of flesh and intravalvular liquid.[3]	None
B	Live bivalve molluscs from these areas must not exceed, in 90% of the samples, 4 600 MPN *E. coli* per 100 g of flesh and intravalvular liquid. In the remaining 10% of samples, live bivalve molluscs must not exceed 46 000 MPN E. coli per 100 g of flesh and intravalvular liquid.[4]	Purification, relaying or heat treatment by an approved method
C	Live bivalve molluscs from these areas must not exceed 46 000 *E. coli* MPN per 100 g of flesh and intravalvular liquid.[5]	Relaying or heat treatment by an approved method

1. The competent authority (= responsible authority) has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons. Harvesting may not be undertaken from areas not meeting the requirements for Class A, B or C.
2. The reference method is given as ISO 16649-3.
3. Regulation (EC) No 854/2004 as amended by Regulation (EU) 2015/2285.
4. Regulation (EC) No 854/2004 as amended by Regulation (EC) No 1021/2008.
5. From Regulation (EC) No 854/2004.

Category III

The results of faecal indicator monitoring of Category III areas should comply with the requirements for such an area and bivalve molluscs harvested from such areas should not contain any hazards at levels that will be deemed to be a risk to human health after long-term relay or post-harvest processing.

The following approach is recommended when there are no existing regulatory requirements:

i. Identify from the risk profile those hazards that may need to be addressed by long-term relay (essentially viral enteric pathogens associated with faecal contamination).

ii. Determine the kinetics of the relay or other post-harvest process to be used in relation to the hazard(s).

> For long-term relaying, this needs to consider the relay method and resulting bivalve density, the seawater temperature and length of relay period.

> For other post-harvest processes, the effectiveness of the method needs to be considered through the bulk of the bivalves, with the worst-case location used to determine the reduction of the hazard over the duration that the process is applied.

iii. Therefore, determine the maximum concentration of the hazard(s) that will consistently yield product complying with the requirements for Category I at the end of the process.

iv. Where there is a direct relationship between the concentration of a faecal indicator and the presence, or concentration, of the hazard, determine the 90 percentile faecal indicator concentration that relates to the maximum concentration of the hazard determined in iii. This faecal indicator concentration may be determined in either water or bivalve flesh, as required for the programme being implemented.

v. Review the results obtained from samples taken post- relay to confirm that the criteria for Category I are met by the relayed product.

Over the review period (at least 24 results), the following should apply:

Either:

> The 90th percentile value for the faecal indicator is less than, or equal to, the value determined from the procedure given in iv above.

> The maximum concentration of the hazard(s) complies with the requirements for Category I.

Alternatively, in the absence of a criterion or criteria based on this approach, the EU criteria for class C (using *E. coli* in bivalve flesh and intravalvular fluid) may be used (see Table 5.2).[26]

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[26] The U.S. NSSP does not contain criteria for areas used for long-term relay. No alternative criteria for assessment faecal coliforms in water can therefore be given.

The use of these criteria may not provide adequate assurance that pathogens of enteric origin are at acceptable concentrations after post-harvest treatment and, unless validation has been undertaken to ensure that this is the case, additional risk management should be considered.

If hazards have been identified in the risk profile that are not reduced to an acceptable level by long-term relay, these should be addressed by separate monitoring and management procedures (either closures or additional processing requirements – see Category IIIb).

The necessary level or length of treatment for some processes to produce bivalves containing hazards at an acceptable level may exceed that which produces product that is acceptable to the consumer (from the viewpoint of consistency, flavour, etc.). The public health aspects must take precedence and, in such cases, the identified post-harvest process may not be appropriate for the bivalve species.

Category IV

Category IV areas either do not meet, or have not been demonstrated to meet, the requirements for Categories I, II or III. They are deemed to be subject to, or potentially subject to, the occurrence, or concentration of, one or more hazards at levels that are not acceptable.

Explanation

The aim of defining the classification level is to ensure that the post-harvest processing requirements that then apply (apart from Category I) reduce the relevant hazards to levels that are acceptable.

5.5   INITIAL CLASSIFICATION

Recommendation

At the end of the period of primary monitoring, assess the faecal indicator results for compliance with the classification criteria used in the programme (see Section 5.4). In addition, the results of any specific hazard monitoring should also be assessed to determine that these show levels deemed to be acceptable.

Other considerations

Where faecal indicator results are compliant with the criteria for a particular classification category, but other monitoring shows the presence of one or more pathogens at a level above that deemed to be acceptable, the classification category for the area should be based on the subsequent processing requirements that will reduce the concentration of the pathogen to acceptable levels.

Where multiple sampling points are specified in a single growing area, the assessment of compliance with respect to faecal indicator criteria or specific hazards should be

based on the point showing the worst results for each determinand. By default, all other sampling points should then comply with the relevant criteria. It is not usually possible to determine whether there are significant differences in results between parts of a growing area, or between seasons, on the basis of primary monitoring due to the limited number of results available.

Examples of the assessment of faecal indictor bacteria data sets obtained from primary monitoring are given in Annex 14.

Assessment of continuing compliance with the initial classification category is determined by a combination of ongoing monitoring, the Growing Area Review process (see Section 7) and interim assessment of monitoring results and growing area status undertaken under expected and/or unexpected event management plans (see Section 8).

Explanation

The results from primary monitoring contribute to the initial classification for an area. It needs to be appreciated that a limited amount of data will be available at this stage and ongoing monitoring may show a different level of indicator(s) or hazard(s) in the growing area.

5.6   DEFINING CONDITIONAL CLASSIFICATIONS

Recommendation

A conditional classification should be considered if the classification status and associated risk is deemed to vary with time (e.g. season) or environmental conditions according to perceived risk of faecal contamination. There are two main alternatives:

> the area is closed when the higher risk applies; or

> the area has a worse classification status, and thus more stringent post-harvest treatment requirements, when the higher risk applies.

The occurrence of results exceeding the relevant classification threshold must be unequivocally predicted by the criteria used to determine conditional classification status.

Other considerations

In applying conditional classifications, it is important that the additional management criteria can be implemented as soon as the higher risk conditions apply. It is also important that the additional management criteria are applied until the risk reduces to lower levels. This means that the specific hazards need to be considered rather than any indicators of those hazards, unless the relationship between indicator and hazard is either absolute or conservative. Conditional classification options include:

Seasonal classifications – Set of months open or at better classification and set of months closed or at worse classification, e.g.

> September to January inclusive: Category I; February to August inclusive: Category II; and

> September to January inclusive: Category I; February to August inclusive: Closed.

Seasonal classifications may also be applied to areas subject to potential or actual contamination associated with boating activity where use of the area for boating is prohibited by the relevant authorities during specified parts of the year.

Rainfall-related classifications – closure or worse classification status after rainfall above a certain level (or a related indicator such as river flow or salinity reduction), e.g.

> rainfall over a 24-hour period exceeding XX mm; or

> salinity measured at the resource is less than XX practical salinity units.

The actual values will depend on the characteristics of the growing area.

Treatment works-related classifications – closure or worse classification status during defined events affecting the level of treatment and thus quality of the effluent, e.g.

> notification of any operation of a by-pass at the treatment works (proportion of the sewage omitting one or more treatment stages);

> notification of the operation of split flows where this is not a normal state for the treatment works (i.e. part of the flow is diverted through the works such that it receives a lower level of treatment than usual);

> notification of loss of disinfection or reduced efficiency of disinfection;

> notification of breakdown at the treatment works or a pumping (lift) station (and thus operation of an emergency discharge); or

> notification of the operation of a storm-related discharge (storm tank overflow, combined overflow, or major surface water overflow).

With respect to classifications based on criteria relating to compliance with faecal indicator levels, the growing area should comply with the classification status during the whole of the open or better classification period. Due to the uncertainty associated with environmental monitoring, any associated risk will be minimized if there are no exceedances of the relevant threshold during the open or better classification period.

Conditional criteria relating to other hazards – the presence, or concentration, of other hazards such as chemical contaminants and biotoxins may be shown to be related to predicable factors that allow the definition of criteria separating periods when a hazard is above and below levels deemed to be acceptable. An example is a seasonal closure for a chemical contaminant where the concentration in a bivalve species varies markedly during the year. However, caution needs to be taken in

applying such criteria as the seasonal nature of the source, or retention by the bivalve, may vary between years and/or may change over time.

The risk associated with the identified hazards should be at a lower level during the whole open/better classification period. Therefore, re-opening or reduced treatment requirements should be based on a reduction of risk and not just reduced indicator levels. An appropriate *in situ* relay period should be applied in order to ensure that the specific hazard(s) is(are) reduced to levels that do not pose a risk to human health.

It is also important that the criteria triggering the conditional closure or reclassification are relatively simple and thus easily assessed and understood and that the associated risk management actions are also simple and easily communicated. Lastly, if conditions in the growing area are such that the conditional criteria are triggered frequently, there is a need to review whether those criteria are appropriate and whether the approach is properly reducing risk from the associated hazard(s).

Explanation

The application of conditional classifications may assist the industry to utilize areas that are otherwise closed or at a classification level where the processing requirements make the area commercially unviable. However, it is important that the conditional criteria are applied to manage the hazard(s) that apply and not just indicators of those hazards.

5.7   DETERMINATION OF THE SIZE OF BUFFER ZONES AROUND DISCHARGES (OR OTHER IDENTIFIED SOURCES)

The following approaches to determining buffer zones around sources of contamination are recommended unless:

i.   the level of the relevant hazard(s) has(have) been determined as being acceptable for the classification level/area status concerned across the entire growing area; and

ii. evaluation of the pollution source(s) and, hydrological and meteorological conditions, determines there is adequate distance from the source.

5.7.1   BUFFER ZONES AROUND POINT SOURCES

Recommendation

Determine the average loading of the hazard (or indicator) in each discharge, together with the variability in the loading over time. Such variation may have both seasonal and diurnal components and may also be affected by weather conditions, such as rainfall and ambient temperature. The loading is the amount of the hazard or indicator discharged per unit time (e.g. *E. coli* per day). The concentration of the

hazard or indicator is then determined at one or more points in the assessment area, by means of one of the following procedures:

> simple dilution calculation;

> salinity studies;

> drogue studies;

> dye tracing studies;

> use of other tracers (fluorescent particles, bacterial spores, bacteriophages); or

> hydrodynamic modelling.

The following factors may be useful when considering or determining the appropriate size of a buffer zone:

i.   volume flow rate of discharge(s);

ii.   location of discharge(s);

iii. performance of treatment works (where applicable);

iv.   microbiological quality of the effluent;

v.   decay rate of contaminants;

vi.   wastewater dispersion and dilution;

vii. time of waste transport to the bivalve resources;

viii. location of bivalve resources; and

ix.   classification of adjacent waters.

Further considerations relating to these factors are given in Annex 10.

Other considerations

In principle, separate studies need to be undertaken for each point source that potentially has an impact on, or within, the assessment area. However, an initial qualitative or semi-quantitative assessment may identify one or a small number of sources as having the greatest effect on the concentration of the hazard or indicator within the assessment area.

The acceptable concentration of the hazard (or indicator of that hazard) at the boundary of the buffer area needs to be defined so that this can be used in the assessment process. Where an adjoining growing area is to be harvested for bivalves to be consumed without any processing, the acceptable concentrations for all hazards at the buffer zone boundary must be those stipulated for Category I areas. If the adjacent growing areas are Category II or III, then the maximum concentrations stipulated for such areas should be used on the basis that the concentrations of the hazards are acceptable are the prescribed treatment for the category of area. Where more than one category of growing area abuts the buffer zone, the most stringent requirement should be used in defining the buffer zone (e.g., if there are Category I

and Category II zones, use the requirements for Category I). If the classification for an adjoining area changes, and there are no other adjoining areas to be taken into consideration, the extent of the buffer zone should be re-determined.

Where there are uncertainties in the data available for determining the size of a buffer zone, it should be sized conservatively (i.e. larger) in order to be protective of public health. The size may be reduced if and when uncertainties are addressed, e.g. more robust data is available on the transport, dispersion and dilution of the hazard(s) being addressed.

To determine if a treatment works or collection system discharge to the catchment draining to a receiving water that may potentially impact a bivalve mollusc growing area, in the absence of a performance history of the treatment and collection system or influent and effluent quality, a conservative approach would be to assume a worst case raw sewage discharge. In the absence of data, the U.S. NSSP recommends assuming that a level of 1.4×10^6 faecal organisms per 100 ml of water is assumed for a raw sewage release that a 100 000:1 dilution would be needed to meet the target used in the NSSP of 14 faecal coliform per 100 ml of water. The same value may be used for the target number of *E. coli* in water. If dilution analysis determines that the location of the discharge is such that the dilution of effluent would be greater than 100 000:1 then the treatment works could be considered located outside the zone of influence on the bivalve mollusc growing area. Different dilution ratios may be applied depending on the known concentration of sewage, provided that the water quality objective of the downstream harvest area is met.

In areas where the treatment works discharge dilution are less than 100 000:1 and/or a raw sewage release results in *E. coli* levels in the growing area above the established standard, the waters may be classified as Category II, and conditional management may be considered. However, this strategy would only be recommended for highly efficient treatment works that are well monitored to detect malfunctions and changes in effluent quality, and when the competent authority has the resources to effectively administer and monitor the conditions of the growing area.

A minimum dilution around a treatment works recommended in the NSSP is 1 000:1 provided that the treatment works can provide greater than a 2-log reduction. Again, the application of such a value will depend on the hazard(s) identified as being relevant to the area, the category of the adjacent growing area, whether a conditional classification is applied, and any target hazard or indicator concentrations established for the growing area programme.

Whichever approach is taken, monitoring at the boundary (or the nearest bivalve resource, if undertaking a bivalve monitoring programme) should comply with any target values for the hazards and indicators relevant to the category for the area.

Explanation

It is a basic principle that it is sensible to keep sewage and food separate from each other. Monitoring in the vicinity of point sources may not properly reflect the risk due to high variability arising from inadequate mixing of the hazard in the water column.

Buffer zones defined around the point sources reflect the fact that the hazard(s) may be present at levels that are not acceptable. Such point sources are usually sewage discharges. However, they may be other sources of faecal contamination (e.g. contaminated watercourses assessed at the tidal limit, or abattoir effluents) or sources of other hazards such as chemical contaminants or radionuclides.

5.7.2 BUFFER ZONES AROUND MARINAS AND OTHER CONCENTRATIONS OF BOATING ACTIVITY

Recommendation

Faecal indicator bacteria – For water adjacent to a marina or other concentration of boating activity, the maximum number of boats that may use the marina or area should be determined (e.g. by the number of slips or moorings), an approximate occupancy rate per boat should be estimated and the total faecal loading for the marina then estimated on the basis of the total number of people multiplied by 2×10^9 faecal coliform/*E. coli* per day per person. Dilution should then be determined around the boundaries of the marina or boating area using the average depth. The boundary of the buffer zone is set where the indicator is estimated to reach an appropriate target value. The target used in the U.S. NSSP is 14 faecal coliform per 100 ml of water. The same value may be used for the target number of *E. coli* in water. A different appropriate limit may be used instead: for example, based on criteria determined locally for the appropriate category of growing area (see Section 5.4).

Hazards – For a hazard of faecal origin, use the same approach as for faecal indicator bacteria, with an estimate of the loading per person and an acceptable level at the edge of the buffer zone substituted for the faecal coliform/*E. coli* values given above. For enteric pathogens, the loading per person will vary more greatly than for faecal indicator bacteria (from zero to very high numbers) and so an appropriate value may be difficult to determine. In principle, the average number excreted per infected person per day will be multiplied by the proportion of infected persons expected under a realistic worst-case situation.

For chemical hazards associated with boating activity, e.g. chemical contaminants from anti-foulants, or fuel spillage/leakage, an estimate should be made of the likely concentration within the marina/boating area. Dilution should then be determined around the boundaries of the marina or boating area using the average depth and an appropriate target value used to determine the boundary for the buffer zone.

Explanation

Concentrations of boating activity may result in significant input of faecal contamination, antifouling agents and/or petrochemicals to the marine environment in the vicinity. Inputs are usually relatively random with respect to location and time and thus difficult to reflect within a monitoring programme. A buffer zone approach is used to estimate worst case situations in order to ensure that relevant hazards are at a level deemed to be acceptable at the boundary of the adjacent growing area.

5.8   DOCUMENTATION OF CLASSIFICATION STATUS

Recommendation

The classification status of the growing area should be formally documented. The documented status should be updated when necessary following a Growing Area Review (see Section 7.5) or the outcome of actions under an event management plan (see Section 6.2). The classification status should be formally published, preferably in a form by means of which it is available to all stakeholders.

Other considerations

The date of the initial classification determination should be recorded along with the date(s) of any revision(s) to that status. The justification for the status should be clear, either by traceability to the relevant Growing Area Assessment or Growing Area Review, or by separate documentation.

Explanation

It is important that staff of the responsible authority, the control authority responsible for surveillance and enforcement, harvesters, wholesalers and others potentially involved in trading or using the resource from the growing area are aware of the classification status of the growing area, and when this status changes. This is to ensure that the appropriate post-harvest treatment (where necessary) and enforcement can be undertaken. These are best achieved by formal documentation of the status and publication of that status so that it is available to all interested parties.

It is necessary for the justification for the classification status to be clear to support reviews of the status. This is ensured by ensuring that there is a link between the link on the status of the growing area and the information and data that formed the basis for that decision. This also helps to provide confidence in the classification programme.

CHAPTER 6
GROWING AREA MANAGEMENT

6.1   CAPABILITY OF THE RESPONSIBLE AUTHORITY(TIES)

Recommendation

The responsible authority should have the ability and resource to monitor and assess for changes affecting the status of growing areas, with respect to the identified hazards. This includes the ability to properly apply the criteria affecting conditional classifications and the ability and resource to undertake ongoing surveillance activities in the growing area and any necessary investigations and management actions (e.g. enforcement of no harvest during closures or enforcement of increased processing requirements). These capabilities may be shared with other regulatory bodies where this is defined in regulations or binding agreements (e.g. memoranda of understanding).

Other considerations

The responsible authority should explicitly publish the boundaries and classification status of each growing area together with any criteria for conditional classifications or the imposition of other closures, and when such closures or reclassifications are in effect. In addition, the classification status, closures or reclassifications should be communicated directly to harvesters, wholesalers and other stakeholders. This includes clearly identifying that no harvesting may take place during closures, or what additional processing requirements are necessary if reclassification takes place. There should also be clear communication to those parties when a closure has been lifted. Where there is a permanent closure due to a high risk from enteric pathogens (i.e. Category IV), or ongoing levels of biotoxins or chemical contaminants above acceptable limits, the authority(ties) should be able to ensure that harvesting does not take place on an ongoing basis.

Where a growing area is subject to a permanent closure due to a defined hazard or hazards, the authority may determine that periodic or intermittent monitoring at a

low frequency is justified in order to determine whether the level of the hazard(s) has changed: this may allow use of the bivalve resource if the level(s) fall to those deemed to be acceptable. The authority(ies) should undertake additional monitoring to confirm the new status if the low frequency monitoring has shown such a reduction in levels. A Growing Area Review should then be undertaken prior to (re-)classification, monitoring and harvest (see Section 7).

Explanation

It is necessary for the responsible authority(ies) to make effective decisions when the risk associated with one more hazards is deemed to have increased to unacceptable levels, to communicate those decisions to all stakeholders, and to be able to enforce any measures (e.g. closure of growing areas or additional processing requirements) in order to ensure that the consumers are not exposed to unsafe products. In addition, resource spent on a sanitation programme is wasted if the responsible authority(ies) are not capable of effectively managing the growing area(s).

6.2   EXPECTED- AND UNEXPECTED-EVENT MANAGEMENT

Recommendation

Management plans should be established for the growing area prior to, or at, the stage of initial classification and should be made available to all stakeholders.

Other considerations

Category I, II and III growing areas all need management plans. The content of the management plans will vary depending on several factors, including the complexity of the area with respect to whether any conditional criteria are applied, the number of fisheries and harvesters operating in the area, and the number and type of sources of contamination (where relevant).

Events may be expected (with detailed plans of the conditions under which they apply and the management action to be taken) or unexpected (where a wider range of events may be encountered, potentially with a range of possible management actions). Unexpected event management is more complicated and may involve investigative action and a risk assessment. Depending on the circumstances, these may be undertaken prior to management action or after a precautionary closure. There is a need for the responsible authority to consider whether an event that may usually be handled as an expected event (e.g. a conditional closure following rainfall) should be managed as an unexpected event. For example, a conditional closure following rainfall should be considered as an unexpected event if the weather conditions that gave rise to the closure were extreme or if monitoring for specific hazards has shown continuing levels of public health concern. In such cases, the lifting of the harvesting area constraints should be undertaken on the basis of a documented risk assessment by the responsible authority (see Section 6.2.2).

There are some hazards for which laboratory analysis is expensive (e.g. dioxins, some pesticides). In such cases, management plans may need to address a potential risk identified in the Risk Profile without the benefit of regular monitoring. This will usually require a precautionary approach to be applied, with targeted monitoring in response to indications of increased risk.

Many elements of a management plan may be common to several growing areas, or even all growing areas within a country. It is therefore possible to produce a generic plan or template that is modified for each growing area. However, in such cases, it is important that all aspects specific to the individual growing area are addressed. If this is not done, the resulting plan will be unlikely to be effective.

Explanation

The purpose of establishing management plans is to ensure that, when the level of one or more hazards may exceed acceptable levels, effective assessment, communication and risk management actions are undertaken to determine and, mitigate the potential risk. It therefore allows a planned, rather than *ad hoc*, response and should increase the speed and effectiveness of that response and usually reduces the required level of resource necessary to produce an acceptable outcome.

6.2.1   EXPECTED-EVENT MANAGEMENT

Recommendation

A management plan should be established to cover those events identified in the growing area assessment as being likely to:

> occur in the vicinity of the growing area, to have known effects on the level of an indicator or hazard in the growing area; and

> be predictable or detectable in time for assessment of the changed status and implementation of appropriate management actions before harvest of affected bivalves has taken place.

Expected events include those relating to the criteria associated with conditional classifications. There should also be a plan for investigating unexpected results (very high or very low) obtained from monitoring. Higher-than-normal results may occur due to contamination events, environmental factors or laboratory error, while lower-than-normal results may be the result of laboratory error.

Other considerations

If there are any uncertainties in the criteria used to define the more contaminated period, the conditions for the imposition and lifting of harvesting should be defined to err on the side of public health protection. If indicator monitoring is used to support re-opening rather than direct monitoring of the hazard(s) itself(themselves) (e.g. faecal indicator bacteria to represent enteric pathogens) then adequate allowance should be made for differences in natural depuration of the indicator(s) and the

hazard(s). Where possible, re-opening should be supported by explicit monitoring for the hazard(s).

Elements to be defined in the management plan are:

> the unique identifier of the growing area;

> the boundaries of the growing area (preferably in both text and map or chart form);

> the conditions under which an expected event is defined to occur;

> the authority responsible for defining those conditions;

> the management action to be taken when those conditions are met;

> where relevant, communication arrangements with the wastewater treatment works management (or the environmental regulator);

> the management action to be taken if a monitoring device associated with the determination of those conditions (e.g. rainfall gauge, sewage overflow alarm) fails;

> the authority responsible for ensuring that the management action is taken;

> the conditions under which the event is deemed to have ended;

> the authority responsible for defining when those conditions are met;

> the time period after the end of the event after which the associated management action is rescinded (if relevant);

> communication arrangements, contact details and any communication cascade – for all relevant authorities, harvesters and other relevant stakeholders; and

> what action to be taken if there is a failure in management action, notifications or other communication.

Other relevant stakeholders may include fisheries authorities, environmental regulators, industry bodies, bivalve mollusc wholesalers and direct customers (e.g. local restaurants).

Possible management actions for Category I areas are closure, institution of post-harvest processing requirements without reclassification or reclassification.

Possible management actions for Category II and III areas are closure, a greater level of post-harvest processing requirements (e.g. heat treatment under controlled conditions rather than depuration) without reclassification or reclassification.

For some bacterial pathogens, especially naturally occurring marine vibrios, management actions may include a time or temperature control requirement between harvest and any subsequent processing, packing and/or transport.

It needs to be considered whether the selected management action is intended to address a greater risk of a hazard than is already addressed within the classification system (e.g. an elevated risk from enteric pathogens as indicated by high faecal indictor results) or whether it is an additional risk, e.g. presence of biotoxins or

naturally occurring marine vibrios at levels above those deemed acceptable. Periods after an event during which closure or increased processing requirements still need to be applied should be defined on known depuration rates of the hazard(s) from the species of bivalves in question. This may need to be validated in the country, region or growing area in order that the appropriate environmental conditions apply and can be occasionally verified by monitoring of hazard after an event. For hazards that have severe health outcomes (long-term incapacity or death), it is preferable to undertake such verification after each event.

CAC (2010a) and CAC (2012b) contain recommendations on procedures to address risks from vibrios and viruses respectively. The U.S. NSSP contains detailed provisions regarding the assessment and control of vibrios (FDA, 2015). With respect to enteric viruses, CAC (2012) recommends that

> "If there is evidence that the area has been affected by human sewage, testing of water or bivalve molluscs for the presence of indicators of faecal contamination and/or NoV or HAV, as determined by the competent authority or an equivalent approach to ensure safety, may be an option prior to re-opening."

With respect to *V. parahaemolyticus* and *V. vulnificus*, CAC (2012) advises that, for an area where controls have been deemed appropriate for one or both of these bacterial species, control measures should be instigated

> "when levels of *V. parahaemolyticus* and/or *V. vulnificus,* or environmental parameters exceed testing or monitoring criteria that are based on risk assessment, if applicable".

For certain hazards, e.g. vibrios and biotoxins, it may be appropriate to have a management plan covering a number of adjacent growing areas if these tend to be affected by events to the same extent and at the same time. In this case, a separate management plan should be defined for the hazard(s). The plan should contain the same type of information that is in a single growing area management plan but should specify the identifiers and boundaries for all of the growing areas. The relationship between such a management plan and any specific for a single growing area (e.g. related to classification status and microbiological hazards) should be clear. At a minimum, there should be cross-reference among each plan.

Areas that are specifically prohibited, including buffer zones between hazard sources (such as sewage discharges) and a classified growing area should have a management plan. At the minimum, this should specify the intended surveillance activity in addition to the area identifier and boundaries.

A template for an expected-event management plan is given in Annex 15.

Explanation

Expected events, by their nature will occur in an area at some point and the nature of the events can be defined. It is therefore possible to define the event, the event trigger(s), the subsequent actions and the event closure trigger(s). Establishing a

written plan and making sure that all interested parties are aware of the plan should ensure that any associated risks are managed properly.

6.2.2 UNEXPECTED-EVENT MANAGEMENT – EVENT THAT FALLS OUTSIDE YOUR RANGE OF DATA

Recommendation

A management plan should be established to define the investigation and assessment of unexpected events, together with the communication of information about the event and the management actions deemed to be necessary in response to the event.

Elements of the plan of management for unexpected events include:

> how to identify when an event has occurred (including communications with other responsible bodies, e.g. environmental regulators and sewage works operators); and

> Risk Assessment to determine whether risk management action is needed (and what form that should take).

This will vary with the individual event and the hazard(s) involved but may include:

> growing area investigation;
  - source (if not known);
  - visual or other evidence of extent affected; and
  - is this event continuing?
> epidemiological investigation;
> sampling and analysis (relevant to the hazard(s)). sampling may be undertaken in a targeted manner, concentrating on a specific source, or may be spread through an area to yield information on the extent of contamination;
  - sampling will usually be undertaken more frequently than for routine monitoring;
  - results of sampling may also be also be used to support review, revision or lifting of controls (including closures);
  - it may be appropriate to undertake a broader suite of analyses on the samples than for routine monitoring, e.g. for events related to faecal pollution it may be relevant to undertake testing for MSC or enteric pathogens in order to support the risk assessment and event review process. Where non-routine analyses are undertaken, it is important to be able to relate any results to the expected levels under non-event conditions. This may necessitate some monitoring in advance to support event management activities;
  - CAC (2012) identifies that

"When there has been a bivalve mollusc-borne outbreak caused by an identified pathogen such as NoV or HAV and the area has been closed,

viral testing of the bivalve molluscs or an approach consistent with the requirements of the competent authority should be used as part of the process of re-opening the affected area to ensure product safety, using either standardized methods or alternative validated methods. Other conditions, including meeting the sanitary survey requirements, should also have been satisfied as a condition of re-opening the area. Ideally these should include the identification of sources of pollution/contamination, and prevention of future contamination events."

> information from industry on current harvest and product destination;

> assess all relevant information in relation to risk profile and growing area assessment (e.g. significance of source in relation to others, knowledge of bathymetry and hydrodynamics);

> Risk Communication (see also Section 6.3);

Collaboration between different authorities (need for formal Agreements, or Memoranda of Understanding);

Communication with harvesters (commercial and/or recreational), wholesalers (and other potential purchasers of commercial harvest);

> Risk management:

> decision tree to help decide upon relevant harvesting area constraints based on the risk assessment process;

> patrol activities to ensure application of the harvesting area constraints;

> control of traceability of product (for recall if determined necessary);

> surveillance in dispatch centre or process establishments;

> follow-up:

> review of the initial risk assessment (during and after the event);

> when deemed appropriate by such a review, revision or lifting of controls (including closures);

> as far as possible, the criteria for lifting additional controls (or a closure) should be defined in advance so that these do not have to be considered in the middle of an event;

> it is important that the lifting of controls is based on the risk from the actual hazard rather than a general indicator of the hazard;

> communication of outcomes; and

> inclusion of occurrence and outcome in the growing area review process (See Section 7) may contribute to ongoing monitoring and control).

It may be possible to link the management plan to others that are already in place, e.g. those for oil spills at sea.

Other considerations

In this context, the term "unexpected event" relates to an occurrence, either affecting a potential source of a hazard, or environmental factors affecting how sources affect a growing area, where there are no signs that are predictably related to the occurrence of the events. Table 6.1 gives examples of unexpected events and the hazard groups that might be affected by the event.

TABLE 6.1 **EXAMPLES OF UNEXPECTED EVENTS**

EVENT	HAZARD GROUP TO BE CONSIDERED
Abnormal weather conditions – severe storms (hurricanes, tornadoes, typhoons, etc.)	Bacterial (including vibrios), viral and protozoal pathogens, chemical contaminants associated with sediments
Abnormal weather conditions – exceptionally warm weather	Vibrios, possibly other bacterial pathogens, biotoxins
Abnormal weather conditions – exceptionally cold weather	Norovirus
Failure of sewage treatment plants, breakdown in sewage pumping stations, breakage of sewerage system	Bacterial, viral and protozoal pathogens of human enteric origin
Spills of animal waste (e.g. from slurry storage systems)	Bacterial, viral and protozoal pathogens of animal enteric origin
Outbreaks related to established (*Salmonella, Vibrio*, virus) or "novel "or emergent pathogens (e.g., *Cryptosporidium parvum*)	Pathogen associated with outbreak (reaction to the event may need to proceed before, or in the absence of, confirmation of the causative pathogen)
Illness associated with biotoxins	Biotoxin group associated with illness
Oil spill or discharge containing other chemical contaminants (e.g. spill from industrial plant, spill of water from mine)	Associated chemical contaminant(s)
Elevated indicator or hazard result, e.g. *E. coli* result above the limit for the current classification of the growing area or above some other predefined action limit	Depends on the indicator or hazard that has given the high result.

A template for an unexpected-event management plan is given in Annex 16.

Explanation

As with expected events, having a management plan in place for unexpected events facilitates a more effective response and potentially saves resource.

6.3 NOTIFICATION OF INTERESTED PARTIES

Recommendation

All interested parties should be notified promptly when a growing area is closed, when the harvested bivalves are to be subject to a higher level of post-harvest

treatment or when export to certain countries will not be allowed. The interested parties should also be notified promptly when the growing area is re-opened or other additional controls are withdrawn. The reason for the application or lifting of a closure or additional requirements should be identified in the notification.

The responsible authority should consider public health advisory warnings for recreational harvesters when an area is determined to be a high public health risk.

Other considerations

Interested parties will include all relevant staff of the responsible authority; staff of other authorities having a role or interest in the growing area sanitation programme; known harvesters; and wholesalers and processors that may receive bivalve molluscs from the growing area.

Means of notification include: e-mail, telephone, Short Message Service (SMS; text message), web-page information displays, posters (at the growing area and at landing points), newspaper advertisements, television or radio advertisements, mailed letters, The chosen method(s) should be relevant to the receiving party, e.g. are the target recipients known persons with defined contact details (such as officials or licensed harvesters), or are they not necessarily known (e.g. unlicensed public commercial fisheries, artisanal or recreational harvesters). More than one means of notification may be needed to ensure that all intended recipients receive the information.

Explanation

Effective risk management ensuing from reclassification or expected or unexpected events requires that all parties having an interest in the growing area sanitation programme are made aware of any change in the status of the growing area, and associated change in any post-harvest processing requirement, and again when the situation has returned to normal (if appropriate).

Providing information on the reasons for the risk management decision helps the interested parties understand the situation and may improve compliance with the requirements. Where the reclassification or event is potentially associated with contamination, the identification of the issue and potential (or actual) sources may assist in progressing corrective action.

6.4   GROWING AREA SURVEILLANCE (PATROL AND ENFORCEMENT)

Recommendation

The responsible authority should have a written plan detailing the surveillance (patrol and enforcement) activities to be undertaken in the growing area both during periods when the area is open and when it is closed (if appropriate).

Other considerations

The surveillance plan should include the following details:

> growing area identifier;

> growing area boundaries (in both text and map or chart form);

> classification category;

> any conditional classification criteria that apply;

> details of licensed harvesters (if appropriate); and

> known or approved landing points.

The nature of the surveillance to be undertaken during each patrol should be specified (e.g. observation of fishing activity and its location, checking species harvested, associated records, landing location(s), destination with respect to processing or packing).

The frequency of surveillance should be determined on a risk basis depending on the nature of the bivalve mollusc resource, the status of the area (open, closed and classification category) and whether there has been previous illegal activity. Regulation may stipulate the minimum frequency of surveillance. The nature of surveillance should be related to the type of fishery: e.g. land-based patrol may be relevant to intertidal fisheries and boat-based to subtidal. However, this will also depend on the accessibility of the area, nature of surrounding terrain, etc. A complete surveillance system will also include audit at landing points and receiving premises (packing and processing plants).

Where possible, surveillance activities should be coordinated with other relevant agencies, e.g. those enforcing fisheries regulations and those responsible for inspecting processing and packing plants.

Traceability is improved substantially if there is a requirement for tamper-proof seals to be fixed on containers/bags of harvested product in the growing area and for this to include a durable label (tag) specifying, in an indelible manner, the name of the harvester, the growing area identifier, the growing area classification category and status, and the intended destination.

Further considerations relating to developing a surveillance plan to detect illegal harvesting are given in Annex 17.

Explanation

Establishing a written risk-based surveillance plan improves the targeting and therefore effectiveness of surveillance activities within the harvestable resource. Effective surveillance adds to the confidence in the sanitation programme for the area.

CHAPTER 7
GROWING AREA REVIEW

This part of a sanitation programme comprises a review of the ongoing relevance of the risk profile and growing area assessment, together with an assessment of monitoring data, in order to determine whether the classification status and management plans need to be revised.

The review process is important in identifying changes within the area that might affect the range of hazards that are of concern, and in identifying changes in the level of risk from identified hazards. Hay, McCoubrey and Zammit (2013) identified that there was a tendency to assume that such changes had not occurred with respect to a growing area and that this was a key factor in sanitation programme shortfalls that led to bivalve-associated viral outbreaks in Australia and New Zealand.

7.1   DEFINED PERIOD FOR ROUTINE REVIEWS

Recommendation

One or more review periods should be defined for the review of each of the following elements:

> Growing Area Risk Profile;

> Growing Area Assessment;

> monitoring data;

> classification status; and

> management plans.

The review periods will normally be:

> short term (e.g. annual);

> medium term (e.g. every 3 to 5 years); or

> long term (e.g. every 10 years).

The review period may be modified according to a defined assessment of risk for an area.

Other considerations

A review should be initiated at an earlier stage than the end of the scheduled period if:

> information is received that indicates that a source (or sources) previously identified as significant to the growing area (through the growing area assessment or an earlier review) may have changed significantly – e.g., a new or modified sewage or industrial discharge;

> an unexpected event has occurred; or

> the frequency of expected events has been markedly higher than that anticipated during the growing area assessment or last review.

Explanation

Establishing a formal review period ensures that the growing area boundaries, sampling plan, classification and management plans are updated as changes occur in an area. Modification of the review period according to risk can enable prioritization of resource with less frequent reviews undertaken for areas with no significant sources and low variability in the monitoring data. However, a review needs to be undertaken outside of the normal cycle if information or monitoring data indicates that the levels of inputs or illness risk have changed.

7.2   CONTENT OF REVIEWS

Recommendation

The expected content of the periodic review should be defined with respect to the range of information and data to be considered, the assessment to be undertaken, and the elements of the sanitation programme for the growing area that might be modified according to the outcome of the assessment.

If more than one review period has been defined for one of the elements of the programme, the level of detail of the review for each period may differ. For example, an annual review could consider the additional monitoring results over the previous year as well as relevant information obtained with regard to any changes in pollution source status (see Section 7.3) and the occurrence of expected or unexpected events and the outcomes of these. Reviews undertaken at a greater interval should include a more comprehensive updating of the information in the original growing area assessment, and intervening reviews, as well as a review of the monitoring data and occurrence of expected or unexpected events. More thorough reviews should include consideration of each element of the risk profile and desk elements of the growing area assessment, and a new shoreline survey. The geographical area to be covered with respect to pollution sources should be the original assessment area and not just the growing area itself.

The outcome of each component of the review should be documented. Any changes should be compared to the information in the previous growing area assessment so that the history of the programme for the growing area, together with any changes over time, can be clearly tracked so that the reasons for any changes are evident. The review may include a new shoreline survey.

A template for a Growing Area Review is given in Annex 18.

Other considerations

Both types of review should address the following aspects:

> sampling, sampling transport and laboratory analysis.

> > was sampling targeted appropriately?

> > were the samples tested at appropriate laboratories? And

> > did the laboratories report appropriately? for example, there may be requirements for timely reporting of elevated results so appropriate action can quickly be taken.

> monitoring results:

> > were there unusual results that need further discussion? Present all monitoring data, ideally alongside pollution events, e.g. rainfall, seasonal events; and

> > pay as much attention to unusually low results as well as to unusually high results. The nature of results can help identify certain issues, for example ensuring that appropriate sites are being sampled.

> expected and unexpected events:

> > occurrence;

> > reaction to events – was this in accordance with the plans?

> > Is there timely reporting and good cooperation between all parties to any management plans?

> > were there any unexpected events or public health emergencies that need documenting.

> surveillance:

> > review information regarding surveillance activity including that for illegal harvesting in closed areas. Summarize details of activities, findings and actions taken.

The review should also contain Conclusions and Recommendations (see Sections 7.5 and 7.6).

Explanation

The information and data considered for the review are dictated by the characteristics of the growing area, the relevant hazards, the content of the growing area and any events and management actions undertaken in the area. A clear connection between

the information and data considered for the review, the ensuing assessment, and the conclusions provides a clear justification for the recommendations that are the principal outcome.

A major consideration for the review is whether there have been changes to the number, type or operation of wastewater discharges, including those from treatment plants and any intermittent discharges associated with collection systems. It is also important to determine whether there have been changes in other sources of hazards.

The inclusion of an assessment of the operation of key aspects of the programme in the review, such as sampling and laboratory performance, and growing area management, including surveillance activities and event management, provides an opportunity for improvements to the programme to be made where this is considered necessary. Inclusion of post-harvest management, including a review of the appropriate receipt, processing and quality (with respect to required standards) of the harvested bivalves will provide additional verification of the operation of the growing area programme.

7.3   REVIEW OF POLLUTION SOURCES

Recommendation

The level of review of pollution sources should depend on the type of review. For a short-term (e.g. annual) periodic review, it should consist of formally noting, and assessing, any changes in number, type and size of sources of which the relevant authority has been made aware during the period (e.g. by the environmental regulator). For a longer-term periodic review, updated information and data on all pollution sources in the previously identified assessment area should be sought from the appropriate bodies (e.g. sewage processors, environmental regulators). For a review triggered by the occurrence of a higher than expected frequency of expected events, or the occurrence of an unexpected event (such as an illness outbreak), updated information and data should be obtained on the type of pollution sources relevant to the associated hazard.

Other considerations

The new information and data should be compared to that presented in the growing area assessment and, where relevant, the intervening reviews, and assessed with respect to any necessary changes in growing area boundaries, associated buffer zone(s), sampling plan and conditional management criteria (where appropriate).

Explanation

Pollution sources may change over time due to increased population, changes in human activity (e.g. industrial or boating activity), sewage treatment/sewerage improvement schemes, additional sewage discharges or breakages in sewerage infrastructure. Information on some of these may be provided to the responsible

authority as they occur, while others may only be revealed in response to formal data requests or by observation during a new shoreline survey.

7.4 REVIEW OF ONGOING MONITORING DATA

Recommendation

In the absence of any existing regulatory requirements, it is recommended that the faecal indicator results from ongoing monitoring data over the last 3 years should be assessed for compliance with the programme criteria (see Section 5.4).

This may be achieved by:

> use of a formal annual review date, and assessing the previous 3-years' data; or

> use of a rolling 3-year review period (looking back at the last 3 years' data from the date of sampling of the last result received).

The use of a rolling 3-year assessment has the benefit of ensuring that any decisions to change the classification level do not take place a long time after the sampling occasion that triggered that change.

In addition, the results of any specific hazard monitoring should also be assessed to determine whether these show the absence, or concentration of the hazards at levels deemed to be acceptable.

Other considerations

Where faecal indicator results are compliant with the criteria for a particular classification category, but other monitoring shows the presence of one or more pathogens at a level above that deemed to be acceptable, the classification category for the area should be based on the subsequent processing requirements that would reduce the concentration of the pathogen to acceptable levels.

Where multiple sampling points are specified in a single growing area, the assessment of compliance with respect to faecal indicator criteria or specific hazards should be based on the point showing the worst results for each determinand. By default, all other sampling points should then comply with the relevant criteria.

If there is a difference in faecal indicator compliance between sample points, and parts of the existing growing area can be properly managed separately (e.g. if there are separated aquaculture units in the growing area), a statistical test should be undertaken to determine whether the difference in compliance, as well as average levels, is significant. If so, the existing growing area could be divided into two or more growing areas. However, the risk profile and growing area assessment for the existing area will need to be reviewed to determine other considerations such as the hazards to be considered for each new area and whether any buffer zone or conditional classification criteria apply to all of the new areas or just a subset. Each of the new growing areas will need a unique identifier and associated

boundary definitions, together with a separate review report, sampling plan and expected and unexpected management plans. An audit trail should be kept of the relationship of the new areas to the existing area. This will allow cross-reference to the original documentation for the existing growing area (risk profile, growing area assessment, growing area reviews) and avoid the need for replication of the existing documentation for each new growing area.

If there is an apparent difference in faecal indicator results between seasons, a statistical test should be undertaken to determine whether the difference in compliance, as well as average levels, is significant. The pattern, at least in terms of average levels, should be apparent across all sampling points. If these considerations apply, a conditional classification based on season may be applied, with the growing area being closed or given a worse classification in the season(s) yielding significantly worse compliance. However, a seasonal classification should not be considered if the worse season from the faecal indicator perspective does not coincide with a high-risk season for an enteric pathogen determined to be a hazard of concern in the risk profile.

The following approach is recommended where both water and bivalve molluscs are monitored for faecal indicator bacteria, and where no existing regulatory requirements dictate that the results from both matrices are individually assessed for compliance.

> The classification should be determined on the basis of a three-year rolling data set (i.e. the results from the previous three years) of results from the water monitoring programme, with compliance assessed against the 90th percentile standard determined as in Section 5.

> The results of bivalve mollusc monitoring should be used to initiate investigative action when results exceed:

> > The 95th percentile value for data from the specific sampling point;

> > or the following values if insufficient data is available to reliably determine a 95th percentile:
> > - 230 *E. coli* or faecal coliform/100 g for Category I areas
> > - 4 600 *E. coli* or faecal coliform/100 g for Category II areas
> > - 16 000 *E. coli* or faecal coliform/100 g for Category III areas

with the application of short-term management actions if the investigations identify an increased risk to public health.

An example of the review of faecal indicator bacteria data obtained from ongoing monitoring is given in Annex 19.

- While faecal source tracking investigations may be used at a number of points in a bivalve mollusc sanitation programme, a specific use is in the investigation of possible sources of ongoing unexpectedly high faecal indicator results from the monitoring programme. There are a number of relevant publications on available source tracking tools (Meays *et al.*,

2004; Hewitt and Williamson, 2014). The application of next generation sequencing to source tracking has also been investigated (Vierheilig *et al.*, 2015). There is a need for source tracking methods to be properly validated and studies incorporating their use to be properly designed and analysed in order to ensure that the results are meaningful (Stoeckel and Harwood, 2007).

Explanation

The assessment of monitoring data is only one, but a key, element in determining the classification of a growing area. The assessment procedure for the monitoring data should be intended to trigger a change in classification status when a significant change in associated risk has occurred and not in the absence of such a change. It is important to assess indicator data with an understanding of the relationship of this to the hazards that the indictor is intended to represent, as the aim of a sanitation programme is to assess and manage the hazards and not an indicator.

7.5 CONCLUSIONS

Recommendation

The conclusions of the review should relate to the information and data that has been included in the review and, where relevant, should relate these to the relevant information and data in the original Growing Area Assessment and any preceding Growing Area Reviews. The following aspects should be considered.

> Has the monitoring been conducted according to the relevant protocols and sampling plan(s)?

> Have there been significant changes in:

  > the bivalve mollusc fishery;

  > actual or potential hazards; or

  > sources of those hazards?

  For hazards of faecal origin, this means reviewing the information on sources of human and animal faecal pollution given in the previous growing area assessment.

> Have there also been significant changes in:

  > environmental factors, bathymetry or hydrodynamics affecting the significance of the hazards?

Other considerations

It is necessary to determine conclusions from the review that relate to the key aspects of the growing area sanitation programme in order to provide a link between the information and data included in the review and any recommendations that arise from it.

7.6   RECOMMENDATIONS

Recommendation

On the basis of the conclusions, recommend appropriate revisions to one or more of the following:

> Relevant hazards;

>> Has there been a change in hazards deemed to apply to the growing area?

> Growing Area boundaries (and therefore extent of any adjacent prohibited areas, where relevant);

>> Do the boundaries need to be changed to reflect a change in location and/ or extent of the bivalve resource, or the location and impact of the identified sources of contamination?

If subdivision of a growing area is allowed under the regulations, then this should only be undertaken if adequate enforcement can be undertaken of harvest from each subdivision. This will not usually be appropriate if a single bivalve resource crosses the proposed subdivisions. If the divided area is to be classified at different levels, then each part should be given a separate unique identifier in order to assist traceability.

> Sampling plan(s)

>> Does the sampling location, tolerance or frequency need to be amended to better reflect the hazard(s)?

>> Is the spatial or temporal variability in results so high that the number of sampling points/sampling frequency needs to be increased?

>> Is the spatial or temporal variability in results so low that the number of sampling points/sampling frequency could be reduced?

> Growing Area classification status:

>> If based on monitoring data, does this continue to be in compliance with the requirements for the area or has the status of the area changed significantly? Is any change apparent in the monitoring data reflected by known changes in source or impact?

> Conditional classification criteria (where relevant):

>> If a conditional classification has not been applied previously, is there now evidence to support one? If one has been applied recently, is it still justified and do the criteria need to be amended to ensure compliance during the open (or better classification) period?

> Expected-event management plan(s);

>> Do any of the elements of the plan(s) need to be updated?

> Unexpected-event management plan(s);

>> Do any of the elements of the plan(s) need to be updated?

Explanation

The recommendations form the main outcome of the review. The recommendations need to be based on the conclusions in order to ensure that they are robust and justifiable.

7.7 DOCUMENTATION OF GROWING AREA REVIEW

Recommendation

The information and data supporting the review, plus the conclusions and recommendations, should be formally documented. The documentation should also include any updated documentation relating to classification status, sampling plans, event management plans or surveillance plans.

Other considerations

It is important that the recommendations and outcomes of the review (in the form of revised classification status or plans) can be tracked back within the documentation to the conclusions and ultimately to the supporting information and data used for the review.

Explanation

It is important that staff of the responsible authority and other stakeholders can refer to the justification for changes in the growing area sanitation programme that ensue from a review. This is achieved by proper documentation of the review and its outcomes. The documentation process is also necessary to provide a sound basis for any further reviews that are undertaken. Both of these purposes require that there be a clear link from the supporting information and data to the conclusions, and thence from the recommendations and outcomes.

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ANNEX 1

GROWING AREA RISK PROFILE TEMPLATE

TOPIC HEADING	DESCRIPTION
AREA OVERVIEW	> Geographical location > General nature (e.g. offshore, coastal or estuarine)
SCOPE OF RISK PROFILE	> Commercial and, if so, intended market (domestic and/or international [identify target countries]

LEGAL FRAMEWORK	> Current relevant food safety regulations and standards.
	  > National (for each item of regulations or standard).
	    > Number and Title with main requirements relating to the growing area sanitation programme.
	  > International (for each item of regulations or standard).
	    > Number and Title with main requirements relating to the growing area sanitation programme.
	> Jurisdiction, authorities responsible for the sanitation programme.
	  > Local
	    > Main duties
	  > Regional
	  > Main duties
	  > National
	  > Main duties
	> Other official bodies responsible for other regulations relating to growing areas, e.g.
	  > Environmental quality
	  > Animal health
	  > Protected areas
	  > Fisheries regulations
	  > Spatial planning and land use management regulations
	> Interactions between authorities
	  > Formal
	  > Informal

TOPIC HEADING	DESCRIPTION

CURRENT INDUSTRY SITUATION/ CURRENT RESOURCES/ AVAILABLE RESOURCES	> Species of bivalve molluscs to be harvested
	  > Common name
	  > Latin name
	> Location of bivalve mollusc resources
	  > Species (for any species collects the following information)
	    > Location of the species
	    > Harvesting Location
	    > Juvenile production location
	    > Maps
	> Cultivation and harvest practices
	  > Species (for any species collect the following information)
	    > Type of harvest
	     - Wild harvest
	     - Ranching
	     - Aquaculture
	     - Recreational
	    > Location in the water column
	     - Water column
	     - Sediment
	     - Seabed
	     - Rocks
	     - Other structures
	      - Natural
	      - Artificial
	     - Aquaculture equipment
	     - Tide exposure
	    > Harvesting methods
	     - Manual
	      - Hand picking
	      - Diving
	     - Mechanical
	      - Dredging
	      - Stripping
	       - Lines
	       - Bouchots
	     - Lifting
	      - Lines
	      - Nets
	    > Relaying or wet storage activities
	    > Distance to landing sites from growing areas
	    > Bivalve mollusc industry capability
	     - Harvesting
	     - Transport
	     - Processing
	    > Seasonality of harvest
	     - Annual
	     - Seasonal (specify months)
	    > Growing Area production capability
	> Seasonal water and air temperatures
	  > Water
	  > Air

TOPIC HEADING	DESCRIPTION
EXTENT OF THE ASSESSMENT AREA	> Boundaries > Geographical name > Maps
EPIDEMIOLOGICAL/ PUBLIC HEALTH DATA	> International > National > Regional > Local > Description of previous outbreaks related to the area

INTENDED USE OF PRODUCTS AND CONSUMING POPULATION	> Societal consumption patterns
	  > Species (for any species collect the following information)
	    > Frequency of consumption
	    > Quantity of consumption
	> Method of presentation, processing and/or preparation
	  > Raw
	    > Live
	    > Half shell
	    > Shucked
	      - With digestive track
	      - Without digestive track
	      - Without other part
	      - Other part used for human consumption
	  > Post harvested processed
	    > Depuration
	    > Short time relay
	    > Long time relay
	    > Cooking
	      - Partial
	      - Total
	    > High pressure
	    > Pasteurization
	    > Freezing
	    > Packaged
	      - Normal atmosphere
	      - Conditioned atmosphere
	> High risk consumers

TOPIC HEADING	DESCRIPTION

OTHER RELEVANT INFORMATION	> Aspects related to contaminated sources
	  > Human activity
	    > Population
	    > Tourist activity
	    > Industrial activity
	  > Sewage discharges
	    > Type
	    > Location (map)
	    > Level of treatment
	    > Quantity of sewage treated (equivalent population)
	  > Concentration of farm animals
	    > Animal
	    > Quantity
	    > Location (map)
	  > Concentration of wild animals and birds
	  > Watercourse
	    > Types (rivers, drains etc)
	    > catchment size
	    > Average flow
	    > Mouth location
	  > Geology
	> Aspects related to the impact of hazard
	  > Topography
	  > Bathymetry
	  > Hydrodynamics
	  > Meteorology
	  > Seawater Temperature and Salinity
	> Existing monitoring data relevant to microbiological and biotoxin hazards

HAZARDS TO BE CONSIDERED	> Microbiological
	  > Bacteria
	  > Virus
	> Parasites
	> Chemical
	  > Organic
	  > Inorganic
	> Radiological
	> Biotoxins

PROGRAMME CAPABILITIES AND CAPACITIES	> Responsible authority
	  > Capability and capacity to undertake work required
	> Laboratories
	  > Distance from sample landing sites (for each relevant laboratory)
	  > Hazard or indicator (for each identified as relevant)
	    > Accredited procedure(s)
	    > Daily numbers of samples to be processed
	    > Response time

CONCLUSIONS AND RECOMMENDATIONS	> Should assessment and monitoring be progressed (go/no go)?
	> If yes:
	  > Hazards to be considered
	  > Boundaries of assessment area
	  > Capability/capacity requirements

• The inclusion of relevant maps will assist the verification and assessment of the information and data.

ANNEX 2

GROWING AREA ASSESSMENT TEMPLATE

TOPIC HEADING	DESCRIPTION
INTRODUCTION	> Growing Area name > Purpose especially hazards to address based on the risk profile.

BACKGROUND INFORMATION	> Summary from Risk Profile
	  > Regulations
	  > Responsible authorities and interactions
	  > Industry and cultivation/harvest practices
	  > Epidemiological information
	  > Shellfish resources, e.g. species, volumes, location
	  > Intended end use/consumption method
	> General Description of the Area (catchment photographs are valuable)
	> Defined growing Area catchment and other Impacting catchments
	> Land use for the greater Catchment
	> History of shellfish programme for the growing area

ADDITIONAL DATA
(EXTRA TO THAT IDENTIFIED
DURING RISK PROFILE
PROCESS)[1]
	> Sources of Contamination
	  > Untreated community discharges
	  > Sewage treatment works
	  > Sewage collection (sewerage) systems
	  > Private sewage treatment works
	  > Other private sewage treatment/handling facilities
	  > Direct human defaecation
	  > Sewage sludge application
	  > Shipping and boating activity
	  > Land use and agricultural activity
	  > Other human activities
	  > Wild animals and birds
	  > Watercourses
	> Geographical, hydrographic, meteorological and other environmental factors
	  > Meteorological Characteristics
	    > Rainfall: Seasonality, intensity.
	    > Winds: Strength, Directions, Seasonality
	    > River Discharges: Volumes, River plumes.
	  > Geology
	    > Soil type
	  > Topography
	    > Runoff potential
	  > Hydrography
	    > Bathymetry
	      - Depths, channels
	    > Channels
	    > Tides
	Type, Amplitude, Tidal exchange rate
	    > Hydrodynamics
	      - Currents: Oceanic, Tidal, Wind Driven, Density Driven, Watercourse Effects, Flood Waters
	      - Stratification
	  > Seawater temperature and salinity

TOPIC HEADING	DESCRIPTION

SHORELINE SURVEY	> Survey Plan – who did what, where, how and when?
	> Identification and evaluation of pollution sources
	    > Domestic Waste
	    > Storm water
	    > Industrial Wastes
	    > Land use and Agricultural activities
	    > Domestic Animals
	    > Water courses
	    > Wildlife
	    > Boat Traffic
	    > Marinas
	    > Guest Houses, Stores, Camps, Houseboats and Fuel Depots
	> Summary of Pollution Sources particularly actual or potential significance

INDICATOR/ HAZARD SURVEY	> Description of survey (if any)
	    > Studies undertaken, e.g.
	        > Water and/or Flesh Quality
	        > Specific hazard(s)
	    > Sample Site location
	> Sample Collection and Transportation
	> Laboratory

DATA ANALYSIS AND ASSESSMENT	> Analytical approaches (e.g. qualitative, semi-quantitative, quantitative) > Data presentation. > Data Analysis Assessment against relevant programme requirements > Development of criteria for expected event management (including conditional classification, if appropriate)[2]
CONCLUSIONS AND RECOMMENDATIONS	> Extent of the classified growing area > Recommendations for primary monitoring[3] > Risk management planning[2]
REFERENCES/ANNEXES	

- This may not be possible until data from primary (or ongoing) monitoring is available)

- The sampling plan(s) may be included here or in an annex (see Section 4.3.1 of the main guidance document for the recommended content of sampling plans)

ANNEX 3

WASTEWATER TREATMENT[1] AND COLLECTION SYSTEM QUESTIONNAIRE[2]

Bivalve growing area(s) potentially affected _____

A3.1   GENERAL ADMINISTRATIVE INFORMATION

	TREATMENT WORKS NAME	
1	Treatment Works Location (latitude/longitude [WGS84] or national grid reference)	
2	Date Visited	
3	Address	
4	Telephone number	
5	Operator(s) Interviewed[3]	
6	Population served	
7	Service connections	
8	Type of influent (circle and approx. % of flow)	a) Municipal _____ b) Industrial _____ c) Combined _____ d) Other _____
9	Date Constructed	
10	Date of any Major Renovations	

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[1]   For raw (untreated) sewage discharges, obtain information for the final pumping station, and associated discharge.

[2]   Complete a separate questionnaire for each works and associated collection system.

[3]   Where access to the plant or interview with operator is not possible, state source of the information (e.g. environmental regulator)

A3.1.1 *Discharge permit criteria[4]*

1	Permit Number	
2	Flow	
3	BOD5	
4	TSS	
5	pH	
6	Faecal Coliform/*E. coli* or other microbial standard	
7	Chlorine Residual, (if Chlorine disinfection is used)	
8	Other (temperature, etc.)	

A3.1.2 *Collection system*

1	Combined Sewage Overflows: Note location of structure and actual discharge point, flows, under what condition they activate, and expected or known number of spills per year. Also note if the associated discharge is subject to any treatment.	
2	Pump Stations: List locations of stations and actual discharges. Note flows (dry and wet conditions) Number of pumps and rated flows; any back-up power	

Note: For systems with several storm or pumping station overflows, include a spreadsheet of the information as an annex.

A3.2 PROCESS INFORMATION

A3.2.1 *Hydraulic Capacity and Treatment*

1	Any influent storage or flow equalization capacity? (describe)	
2	Holding ponds used? (describe)	
3	Holding pond hydraulic retention time?	
4	Holding pond capacity?	
5	Do high tides affect treatment volumes or efficacy? (If so, describe)	

---

4 Where applicable.

6	Do storms affect treatment volumes or efficacy? (If so, describe)	
7	Hydraulic load capacity/design flow? (gallons or cubic metres per day)	

8	Type of treatment (Circle and describe)	> None (raw sewage discharged)
		> Primary settlement
		> Suspended Growth
		    Examples: activated sludge processes, aerated lagoons and aerobic digestion
		
		Wastes being treated flow through and around suspended growth reactor. Microorganisms are removed through settled flocculation for further treatment
		
		> Attached Growth
		    Examples: Trickling filters (also known as trickling biofilter, biofilter or biological filter) and rotating biological contactors (RBC)
		
		Wastes being treated flow over a media in which microorganisms used for treating wastes are embedded.
		
		> Combination
		    Examples: Hybridization of the activated sludge and attached growth systems.
		
		> Other advanced treatment:
		    Examples: Membrane
		
		MBR uses both a suspended growth bioreactor and a membrane filtration process – microfiltration or ultrafiltration
		Membrane treatment works may have a separate secondary treatment followed by a membrane filtration process.

9	Organic loading capacity? (BOD5 value/day)	
10	Full treatment at hydraulic capacity? If not, describe reduction. (estimate % of treatment)	
11	Average daily flow (dry weather)?	
12	Average daily flow (wet weather)?	
13	Peak hourly flow (dry weather)?	
14	Peak hourly flow (wet weather)?	
15	Flow attributable to storm water? (subtract average daily dry flow from average daily wet flow)	
16	Flow Attributable to infiltration? (if known)	
17	Describe various bypass scenarios: (Circle all that apply)	> Raw sewage bypass > Primary bypass without disinfection > Primary bypass with disinfection > Secondary bypass without disinfection > Secondary bypass with disinfection > Other bypass swcenario_____

18	Describe flow or rainfall that historically triggers the bypass. (Provide flow numbers is available)	
19	Any proportion of storm flow stored and returned later for full treatment?	
20	Receiving water name?	
21	Outfall/diffuser description (estimated immediate dilution)	
22	Outfall/diffuser location (latitude/longitude [WGS84] or national grid reference) Identify if discharge location varies under any conditions.	

A3.3   DISINFECTION INFORMATION

1	Is disinfection continuous?	
2	Describe disinfection equipment. Is it tied to residual analyser or proportional to flow?	
3	Can disinfection doses meet peak flow demand?	
4	Is there any redundancy in disinfection equipment?	
5	Type of disinfection (Circle and describe) Skip to pertinent type disinfection questions Chl=Chlorine UV=UV	Chlorine (answer Chl questions) UV (answer UV questions) Other disinfection technology (membrane, ozone, etc.)
Chl	How much Chlorine usage under average flow?	
Chl	How much Chlorine usage under peak hourly flows	
Chl	What is minimum contact time (Ct)?	
Chl	Is effluent de-chlorinated? How? (describe)	
UV	Type of UV system? Make and Model #	
UV	Automatic wipers? Frequency?	
UV	How is dose and wiping frequency determined?	

A3.4   ALARMS AND MONITORING

1	SCADA system present? Describe parameters monitored. (e.g. Note if chlorine residual monitored in SCADA system)	
2	Describe emergency alarms and back-up power capability.	
3	Staffing (night, weekends, holidays)	
4	Frequency of disinfection system monitoring (sample location and method/frequency - continuous or other frequency)	
5	Frequency of faecal indicator sampling (sample location and method)	
6	Alarms for bypasses, overflows, loss of disinfection?	
7	How quickly can operator be notified through alarm system of a bypass, overflow, or loss of disinfection?	
8	Do any of the above events have to be notified to national or local agencies (e.g. environmental regulator, public health protection agency, or authority responsible for bivalve sanitation programme)?	
9	Alarms for pump stations	
10	How does scheduled maintenance of primary, secondary, tertiary, or disinfection units affect effluent quality?	
11	Does scheduled maintenance have to be notified to national or local agencies in advance (e.g. environmental regulator, public health protection agency, or authority responsible for bivalve sanitation programme)?	
12	Is rainfall recorded? If so, what is the location of the rain gauge? Does this differ from that used for classification or management of growing area?	

Note: SCADA = Supervisory Control And Data Acquisition: a system for remote monitoring and control.

A3.5   RECORD REVIEW

Review a sampling of records of flow, chlorine residual, chorine usage, (UV treatment) and microbial indicators. Match several dates with rainfall records to assess rainfall event impact.

Review any records involving treatment interruptions – whether they be reduced treatment levels or the *reduction* or *loss* of disinfection, including power failures, storm events, bypass/overflow events, maintenance work that affected treatment level or disinfection, etc. For each event reviewed, note the date(s), duration, flow affected, reason for problem and records of whom was notified and when that notification occurred.

A3.6   PHOTO DESCRIPTIONS:

1) ....

2) ....

3) ....

4) ....

5) ....

6) ....

7) ....

8) ....

9) ....

10) ....

11) ....

12) ....

13) ....

14) ....

15) ....

Reviewed By _____ Date: _____

ANNEX 4

SHORELINE SURVEY CHECKLIST

A4.1   PRIOR TO LEAVING THE OFFICE

> Programme requirements/Applicable regulation

> Review Central File – past surveys (if any)

> Determine safety/personal security issues

> Make a copy of the past survey report to bring (if any)

> Contact the local authority(ies) to let them know where/when you will be surveying (preferably, invite relevant staff from the authority(ties) to be part of the survey team)

> Leave a plan with the office, where you will be and when you anticipate returning

> Determine boundaries of survey area/maps:

>> Watershed and sub watershed/catchment maps

>> Topographic maps

>> Property ownership/county assessor maps

>> Aerial photos

>> Public works maps of sewered areas

>> Soil conservation maps

A4.2   ACCESS AND PHYSICAL SAFETY
(CHECK AND PLAN RISK-REDUCTION)

> General

>> Determine any barriers to access (using maps/charts)

>> Tides

> Currents

> Weather

> Potential Military activity (including training areas, firing ranges, land-mine areas)

> Potential paramilitary/terrorist activity

> Potential criminal activity

> Land

> Terrain along access route

> Footing along access route

> Intertidal area access (tidal state, substrate)

> Watercourse crossings

> Water

> Categorization of water (to select appropriate type of vessel)

> Number of personnel and planned activities (to identify necessary vessel characteristics)

> Depths (including effect of tidal state)

> Obstructions (rocks, wrecks, debris, etc.)

> Predicted wave height (weather dependent)

> Tides

> Communicate and coordinate with government environmental, health and water quality programmes:

> Enforcement actions in process;

> Enforcement actions pending;

> Location of permitted point source discharges;

> Complaint history and volume;

> Permit history – permit volume, types, problem areas;

> What surveys have been done in the area;

> What reports and surveys already exist; and

> Develop similar survey protocol when possible to share data.

A4.2 OBJECTIVES

A4.2.1 *Evaluate and categorize pollution sources:*

> ***Direct impact***: A pollution source having direct impact is defined as any waste discharge which has immediate impact on the growing area (e.g. sewage or other wastewater discharge direct to the marine or estuarine environment within one tidal cycle's transport distance from the bivalve resource).

> *Indirect impact*: A pollution source having an indirect impact is defined as any waste discharge which reaches the growing area in a roundabout or indirect manner (e.g. sewage or other wastewater discharge that reaches the marine or estuarine environment near the bivalve resource via a watercourse or land runoff).

An attempt should be made to quantify the contaminant loading of any discharge or potentially contaminated watercourse, by measuring the volume flow rate and collecting samples for laboratory analysis (for faecal contamination this will usually be for faecal indicators but could also include pathogens. If the Growing Area Assessment is to consider other hazards (e.g. chemical contaminants, then samples from possibly relevant discharges should be analysed for these).

> Consider prioritizing sources so that resources can be focused;

> Also sample water and bivalves at key points across the intended growing area;

> Locate on field map using GPS – every record needs to have a recorded location;

> Take pictures;

> Complete forms and notes as much as possible whilst on site; and

> Analyse results.

A4.3 COORDINATE WITH LABORATORY(IES)

> Ascertain days and times that they can accept samples.

> Anticipated number of samples (by sample type: sewage, fresh water, sea water, bivalve molluscs, and related determinands)

> Target concentration ranges for different sample types and determinands (to yield quantifiable results rather than less-than or greater-than values) – see Annex A4.13.

A4.4 BRING THE FOLLOWING

> Agency identification:
>   > Business cards;
>   > Credentials.
> Copy of Directions/Communication:
>   > Water resistant GPS (accurate to at least 10 m);
>   > Street maps; and
>   > Cell (mobile) Phone, satellite phone and/or short-wave (or VHF) radio.
> Forms/Supplies:
>   > Forms (preferably printed on waterproof paper);
>   - Shoreline Survey Record;

- Sample request (may be separate forms for seawater, freshwater and bivalves; liaise with the laboratory(ies);
  - Wastewater Treatment Plant assessment;
  - Marina assessment form;
- > Supplies;
  - Permanent markers;
  - Notebook(s) – preferably waterproof paper;
  - Pencils; and
  - Pens.
- > Personal care:
  - > Drinking water;
  - > Food;
  - > Disinfectant gel or wipes (for use prior to handling food or drink);
  - > Appropriate clothing for expected conditions;
  - > Spare set of clothing;
  - > Boots (appropriate for conditions);
  - > Hat;
  - > Gloves; and
  - > Any necessary medications.
- > First Aid:
  - > First Aid Kit;
  - > Insect repellant; and
  - > Sunscreen.
- > Field Equipment:
  - > Water resistant Camera;
  - > Water resistant Flashlight;
  - > Cooler;
  - > Sample bottles (correct size and composition; sterile if for microbiology; labels should be waterproof);
  - > Food grade plastic bags for bivalve samples (with ties or seals); and
  - > Ice/ice packs.
- > Flow measurement equipment:
  - > Calibrated bucket/jug;
  - > 10-metre tape measure/calibrated rod;
  - > Tennis balls or oranges; and
  - > Stop watch.

> Additional items to consider:
  > Flow meter (serviced and calibrated);
  > Water quality probe (conductivity, temperature, dissolved oxygen) (serviced and calibrated); and
  > Dye or charcoal packets.
  > Ensure that all equipment capable of recording or displaying time has been calibrated against a master source, e.g. the GPS).

A4.5   AT A RESIDENTIAL PROPERTY

> Introduce yourself, display credentials, and explain purpose of shoreline survey.

> Be friendly, outgoing, courteous and ask for permission to proceed.

> Understand your legal rights for entering (or not) property.

> Listen to what people say, they may have local knowledge of possible pollution events and pollution sources. Be considerate of their point of view.

> Locate septic (waste water) system.

> For septic tanks, obtain information on sludge removal and maintenance. For cesspits, obtain information on emptying frequency and last date.

> Locate footing drains, interceptor/curtain drains.

> Check for any discharges.

> Bulkheads/tanks.

> Look for seepage – wet areas.

> Drainage channels.

> Nutrients – examples of significant vegetation growth compared with surroundings. Evidence of sewage fungus[5].

A4.6   FARMS

> Document species and number of animals

> How is manure handled or contained?

> Stock access to drainage ditches, sloughs, creeks, and rivers?

> What are the fencing set-backs from shore or conduit to the shore, pasture rotation practices, buffer planting?

---

[5] A mass of filamentous bacteria (primarily of the species *Sphaerotilus natans*), associated with fungi and protozoa, that grows in response to organic nutrients in water

> Determine distance from pasture or manure pile to shore.

> Determine distance from pasture or manure pile to stream or conduit to shore.

> Slurry storage systems and any associated management measures.

> Evidence of recent sludge or slurry spreading or application of other fertilizer.

> Runoff control?

A4.7 WILD ANIMALS

For each species or group (e.g. if flocks of mixed seabirds):

number estimate;

location; and

evidence of accumulations of faecal deposits

A4.8 PARKS/CAMPGROUNDS/BEACHES

> Number of people served

> What type of faecal waste disposal?

> Animals allowed? What type of waste disposal?

> Are they seasonal? Dates of opening or closure?

A4.9 WASTEWATER TREATMENT PLANT (WWTP) AND COLLECTION SYSTEM

Visit WWTP – for STWs fill out STW questionnaire (separate annex); for other WWTPs collect appropriate information on influent (volume and type), treatment levels, effluent (volume, contaminant content), discharge location).

Locate and inspect pumping stations (lift stations) and locations of reported overflows; and

Record, any sewerage system, wastewater treatment plant or apparent discharge locations that were not identified prior to the shoreline survey.

A4.10 MARINA

> This includes ports, anchorages, concentrations of moorings and actual physical marinas and ports.

> Number and types of boats served (include live-aboards)

> Count mooring balls

> Sewage disposal

> Shoreside facilities

> Vessel pumpout facilities, determine if records available of usage

> Other waste disposal

> Waste oil and solvents

> Storm water runoff from parking lots and dry dock areas

A4.11 COMMERCIAL FACILITIES

> Determine nature of the business and type of waste generated. Focus on those that have a potential risk for contamination.

> How waste is handled and stored

> How wastes could contaminate a growing area

> Inspect paved areas/runoff control

> Exterior drains

> Interior drains

A4.12 CLOSEOUT SURVEY AND RECORD KEEPING

> Go over items and observations from inspection

> Report any problems identified to the appropriate national, state (provincial), or local agency with the responsibility to minimize or eliminate pollution sources

> Prepare a written summary of the survey findings (this contributes to the growing area assessment)

> Maintain accurate, legible records

> Unique identifiers for area and properties. Use GPS where possible.

> Keep data consolidated and updated

A4.13 EXAMPLE DILUTION RANGES FOR FAECAL COLIFORM OR
E. COLI TESTING

The following dilution ranges have been developed from experience with shoreline surveys and sewage discharge surveys. For water and effluent samples, the results are expressed as per 100 ml. This approach has been taken from environmental

monitoring programmes (e.g. for recreational waters), where the results for seawaters are usually given per 100 ml, and so results for watercourses and sewage discharges are also given per 100 ml for easy comparison. See Section 4.3.8 of the main text for recommendations on suitable bacteriological methods for these indicators.

Bivalve molluscs: for a five-tube four-dilution MPN assay

Five tubes each containing 1 g of bivalve homogenate (i.e. 10 ml of a 1/10 dilution)

Five tubes each containing 0.1 g of bivalve homogenate (i.e. 1 ml of a 1/10 dilution)

Five tubes each containing 0.01 g of bivalve homogenate (i.e. 1 ml of a 1/100 dilution)

Fives tubes each containing 0.001g of homogenate (i.e. 1 ml of a 1/1000 dilution)

> Nominal quantifiable range: 18 – 180 000 faecal coliforms or *E. coli*/100 g

> Seawater samples for faecal coliforms or *E. coli*: by a membrane filtration method

100 ml of neat

1 ml of neat or 100 ml of 1/100 dilution

> Nominal quantifiable range: 1 to 10 000 faecal coliforms or *E. coli*/100 ml. An MPN method may be used that yields the same nominal quantifiable range

Watercourse samples: by membrane filtration

1 ml of neat (or 100 ml of 1/100 dilution)

0.1 ml of neat ((or 100 ml of 1/1 000 dilution)

0.01 ml of neat (or 100 ml of 1/10 000 dilution)

> Nominal quantifiable range: 100 to 1 000 000 faecal coliforms or *E. coli*/100 ml. An MPN method may be used that yields the same nominal quantifiable range.

Sewage effluent samples; by membrane filtration

0.1 ml of neat (or 100 ml of 1/1 000 dilution)

0.01 ml of neat (or 100 ml 0f 1/10 000 dilution)

0.001 ml of neat (or 100 ml of 1/100 000 dilution)

> Nominal quantifiable range: 1 000 - 10 000 000 faecal coliforms or *E. coli*/100 ml. An MPN method may be used that yields the same nominal quantifiable range.

For ongoing monitoring, or repeat surveys, dilution ranges may be modified based on experience obtained for the area. However, it is important that the dilution ranges used yield results that are of use for the intended purpose.

ANNEX 5

SHORELINE SURVEY PLAN TEMPLATE

NAME (IDENTIFIER) OF SURVEY AREA

_____

SURVEY REASON AND OBJECTIVES

_____

INTENDED DATE(S)/TIMES OF SURVEY

_____

GROWING AREA DETAILS

GROWING AREA	FARM/WILD SITE NAME	IDENTIFIER	CLASSIFIED SPECIES[1]

1. If not already classified, give species to be considered for classification

FARM OWNERS/LICENSED HARVESTERS*

_____
_____

*or indicate if a wild fishery is open to anyone to harvest

CURRENT CLASSIFICATION AND OPEN OR CLOSED STATUS (IF RELEVANT)

_____

CURRENT MONITORING POINTS (IF RELEVANT)

GROWING AREA	FARM/WILD SITE	PROGRAMME M/B/P/C/R[1]	IDENTIFIED MONITORING POINT LAT/LONG WGS84	MONITORING POINT IDENTIFIER

1. M = microbiological; B = biotoxin; P = phytoplankton; R = radiological.

BRIEF DESCRIPTION OF THE SURVEY AREA INCLUDING THE CLASSIFIED AREA(S) (IF RELEVANT) AND BIVALVE FISHERY(IES)

_____

_____

BRIEF DESCRIPTION OF INTENDED SURVEY ROUTE AND MEANS (BOAT AND/OR ON FOOT).

_____

_____

KNOWN SOURCES OF CONTAMINATION (FAECAL, CHEMICAL, RADIOLOGICAL)

Include known sewage discharges, known large aggregations of animals (e.g. large farms), industrial discharges.

NAME OF SOURCE	TYPE OF SOURCE	TYPE OF CONTAMINANT (FAECAL, CHEMICAL, RADIOLOGICAL)	LOCATION LAT/LONG WGS84	NOTES

NAME OF SOURCE	TYPE OF SOURCE	TYPE OF CONTAMINANT (FAECAL, CHEMICAL, RADIOLOGICAL)	LOCATION LAT/LONG WGS84	NOTES

SAMPLING TO BE UNDERTAKEN DURING THE SHORELINE SURVEY

Rationale and approach for sampling during the survey. Approach to be taken if the intended sampling location is not accessible at the time of the survey. Approach to be taken if additional potentially significant sources of contamination observed.

_____

_____

Intended sampling locations (also show on map)

NO.	DESCRIPTION OF LOCATION	LOCATION (LAT/ LONG WGS84)	SAMPLE TYPE	DETERMINAND
1	e.g. From the boil of main city sewage discharge		e.g. Seawater	e.g. *E. coli*
2				
3				

NB: For some determinands (e.g. *E. coli*), a dilution range will need to be agreed with the laboratory for each sample type to ensure that the expected (and relevant) endpoints are obtained (to avoid lots of < or > values.

SAMPLE SUMMARY (TO INFORM LABORATORY(IES)

Usually include allowance of at least 10 percent above the planned sampling locations to allow for additional sampling in reaction to observations during the survey.

*Faecal indicator bacteria (faecal coliforms and/or **E. coli** – identify which)*

	SEWAGE	WATERCOURSES	SEAWATER	BIVALVES

Other (state determinand or determinand group)

EXPECTED NUMBER OF SAMPLES:	SEWAGE OR OTHER DISCHARGE	WATERCOURSES	SEAWATER	BIVALVES

MAP OF SURVEY AREA SHOWING CLASSIFIED AREAS, PREVIOUSLY IDENTIFIED FISHERY LOCATIONS, LOCATION OF KNOWN SOURCES OF CONTAMINATION, INTENDED SURVEY ROUTE AND PLANNED SAMPLING LOCATIONS (BY TYPE OF SAMPLE)

_____

PRACTICAL AND SAFETY CONSIDERATIONS

Tidal state during survey (preferably a tidal curve and table of high- and low-water times and heights) _____

Spring or neap   _____

High or low _____

Sunrise/sunset (state times)

_____

Shore substrate

   e.g. especially identify areas of mud

_____

Other potential access problems

   Fencing, land owners, watercourses that are not safe to cross, etc.

_____

Any other identified safety considerations

_____

NB: The organization managing the shoreline survey may require a separate detailed risk assessment for the fieldwork.

ANNEX 6

SHORELINE SURVEY REPORT TEMPLATE

A6.1   GENERAL DETAILS

Growing Area name (and identifier if known):

Species:

Harvester(s) (where known):

Responsible authority:

Reason for survey:

    Growing Area Assessment

    Growing Area Review

    Growing Area Investigation

Date(s) Surveyed:

Surveyed by:

Existing sampling points (where relevant):

Brief description of survey route:

A6.2   REASON FOR SURVEY:

Growing Area Assessment

Growing Area Review

Growing Area Investigation

    Date(s) Surveyed:

    Surveyed by:

Existing sampling points (where relevant):

Brief description of survey route:

A6.3   WEATHER

Describe weather conditions experienced during survey and note whether there was any rainfall in the previous week.

A6.4   CONTEXT

Fishery

Describe the bivalve fishery, i.e. species, boundaries of aquaculture site or wild harvest resource, method of harvest, seasonality of harvest & any other relevant information.

Sewage Sources

Describe the number and distribution of any dwellings, public facilities (cafes, toilets etc), and sewage discharges in the area.

Seasonal Population

Were there any campsites, caravan parks, hotels, B&B's, holiday homes in the area? If so, describe location and details.

Boats or Shipping

Describe any piers or anchorages in the area. Also note if any boats were observed on the day of the shoreline survey.

Farming and Livestock

Describe the numbers and distribution of any livestock observed in the area. Note if any farms, cattle grids, etc. were observed.

Land Use

Describe the land use (habitation, industry, agriculture, horticulture, grazing, etc.) around the area observed.

Land Cover

Describe the predominant landcover (i.e. woodland, plantation forest, grassland, heath, crops, improved pasture) and any variation in it around the area observed.

Watercourses

Describe the number and distribution of watercourses around the area.

Wildlife/Birds

Describe the distribution and numbers of wildlife types observed during the survey.

Industrial discharges

Describe any industrial discharges seen. Note whether any are known or suspected to have a faecal component (e.g. toilets in a factory discharging into the factory effluent).

Maps

Insert maps showing:

1. Location of observations (may need more than one map for a large area or many observations)

2. Sampling locations annotated with sample identifier and laboratory results (use a different symbol shape or colour for each sample type (e.g. bivalves, seawater, freshwater, effluent) and/or determinand (if relevant). More than one map may be required for each sample type or determinand if many samples were taken.

The same numbering system should be used on the maps and the relevant table of observations or sample results so that the maps and tables are cross-referenced.

TABLE A6.1 **SHORELINE OBSERVATIONS – LINK YOUR NOTES TO GPS POINTS AND ADD TO TABLE BELOW**

NO.	DATE	TIME	LATITUDE	LONGITUDE	ASSOCIATED PHOTOGRAPH	ASSOCIATED SAMPLE	DESCRIPTION
1							
2							
3							
4							
5							
6							
7							
8							
9							

Notes: Photographs referenced in the table can be found attached as Figures A6.1 to A6.xx.

A6.5   SAMPLING

Describe any deviation in sampling from that proposed in the survey plan. Identify how samples were handled and transported, how long after collection the samples were analysed, sample temperature on arrival at the laboratory, and any further information necessary to interpret the sample results such as units.

TABLE A6.2   **WATER SAMPLE RESULTS**

NO.	DATE	SAMPLE REFERENCE	LATITUDE	LONGITUDE	TYPE (E.G.SEAWATER, FRESHWATER, EFFLUENT)	RESULT (E.G. FAECAL COLIFORM/ *E. COLI* (CFU/100 ML)
1						
2						
3						
4						
5						
6						

TABLE A6.3   **BIVALVE MOLLUSC SAMPLE RESULTS**

NO.	DATE	SAMPLE REFERENCE	LATITUDE	LONGITUDE	SPECIES SAMPLED	RESULT (E.G. FAECAL COLIFORM/ *E. COLI* (MPN/100 g)
1						
2						
3						
4						
5						
6						

Photographs

Insert photographs listed in table of shoreline observations & give brief description. Examples below.

FIGURE A6.1   **KIRKCOLM SEWAGE WORKS**

FIGURE A6.2   **SEWAGE OUTFALL PIPE, LOCATION OF WATER**

FIGURE A6.3   **SEWAGE TREATMENT WORKS**

FIGURE A6.4   **DISUSED PIPE**

FIGURE A6.5   **SMALL FRESH WATER STREAM, LOCATION OF WATER**

KEY CONSIDERATIONS IN UNDERTAKING AND ASSESSING A DROGUE STUDY

A7.1 POSSIBLE OBJECTIVES AND BENEFITS OF THE STUDY

> determine the time it may take for pollution sources to reach the growing area;

> determine the direction that the pollution source may travel;

> results can provide some indication of dispersion and area that might be affected;

> results can be used in conjunction with empirical analysis to estimate dilution; and

> results can be used to calibrate and validate model results.

A7.2 INFORMATION TO GATHER (IF AVAILABLE)

> bathymetry data for the water body;

> navigation or any dredged channel locations;

> history of prevailing wind direction and speed – may change with season;

> freshwater inflow – may change due to hydrological events and season;

> structures or other objects that could possibly interfere with drogue; and

> tidal flow information – water level and currents.

A7.3 CONDUCTING A DROGUE STUDY

A7.3.1 *Factors to consider*

Tides

Tides may often be the governing factor in determining pollutant transport in estuaries with large tidal range (e.g. macrotidal >4 metres and potentially mesotidal 2–4 metres) when the volume of tidal water exchanged daily is significantly larger

relative to the volume of freshwater discharged. Spring tides – tides that have the largest difference between high and low water and often when current speeds may be at a maximum would be a preferable time for determining the quickest time of travel for a pollutant source to reach the growing area. This may also be a preferred time for determining the maximum excursion (further distance) a source may travel due to tidal dispersion. Neap tides – tides which have the least difference between high and low water may be a preferable time when determining the longest time a pollutant may reside in an estuary. This may an important consideration in determining how long it may take for a pollutant to be completely flushed out of an estuary after being released, for example, through an exceptional event.

Wind

Wind may often be the governing factor in determining pollutant transport in shallow estuaries with weaker tides that have relatively small tidal range (e.g. microtidal <2 metres). In consideration of a worst-case scenario, wind conditions that would influence the movement of the source towards the growing area should be considered especially in estuaries where wind may be the more dominant factor. In these situations, flexibility may be needed when selecting study dates based more on existing wind conditions than tidal conditions. Note should be given to prevailing wind direction and speed that may change with the season and may help to determine the best timeframe to plan and conduct the study with the best chances of getting the appropriate conditions needed.

Freshwater inflow

Freshwater inflow may influence the transport and the dilution of a source depending on proximity to the source and magnitude of freshwater inflow. Comparing the percent or relative fraction of freshwater inflow in the vicinity of the source to that of the tidal flow in the estuary may help determine the significance of freshwater in the potential transport and dilution of the source. If the percentage of the freshwater inflow relative to the tidal flow is low (e.g. <5 percent) this may indicate that the freshwater inflow may not have a large influence on the pollution source. This percentage may change with changes in freshwater inflow after a rain event and it may also vary with season. If the freshwater inflow is a large percentage compared with the tidal flow this may give an indication that freshwater inflow may have a significant influence on the time of travel and dilution of a pollutant source. For example, periods of higher inflow could result in greater dilution, but it may shorten the time of travel through the estuary. Higher freshwater inflows could potentially provide an additional push to the pollution source and result in an impact further downstream. Additionally, high freshwater inflow in the vicinity of the pollution source could result in stratification, which may also affect the initial mixing and transport of the source.

Tidal depth and stratification

Stratification can occur in waters when masses with different properties form layers that can act as barriers to mixing. Mixing within the water column usually depends upon the degree of stratification with the least dense masses closer to the surface and on top of more dense layers. In waters with little stratification a source may be more evenly mixed from surface to bottom than in waters with large stratification. A pollution source discharging into less dense waters at or near the surface often will remain near the surface and may not mix with waters at a lower depth. Pollution sources that discharge into deeper more stratified waters could potentially stay trapped at a greater depth and mix with water below the surface to a greater extent. For larger sources that discharge below the surface, such as a wastewater discharge, a good visual check is to try to locate near the vicinity of the discharge a plume or "boil" of water at the surface. The boil is often easier to locate on a calm day with little wind at low tide. If a boil cannot be visually located, a small batch release of dye could also verify if the source reaches the surface. If a submerged plume that stays trapped below the surface is suspected then a drogue study may not be a suitable tool for determining the time of travel nor transport of the source.

Bathymetry, navigation channels and structures

The bathymetry and location of navigation channels can provide an indication of the movement of tidal flow in a given estuary. Special attention should be given to structures or other hazards that could cause the drogues to become trapped. For example, shallow areas that may have shoals or could dry may be areas that drogues could become stranded. In locations where this is likely, the use of a small batch release of non-toxic dye maybe be preferable.

A7.3.2 *Types of drogues*

Winged drogues – pro and cons

A simple winged drogue can be constructed with basic materials such as PVC pipe, aluminium metal sheeting as the wings or fins, and held within slits cut along the length of the PVC pipe and held tightly together with hose clamps. Fishing or diving weighs can be suspended a short distance on the bottom end of the drogue to add additional weight. Additional weight may help limit the influence of wind that can affect the float on the surface of the water that is used to visually track the drogue. A line attached to the top of the drogue can be adjusted to various lengths and attached to the float at the opposite end. Winged drogues have an advantage in some circumstances over fruit or other objects that float on the surface as they are less influenced by wind. Additionally, winged drogues can be deployed at various depths within the water column by adjusting the length of the line used. Deploying at various depth helps determine any possible changes in current velocity or direction with depth compared with on the surface.

Fruit or surface drogues – pros and cons

Fruit or other objects that float can also be used as drogues. Fruit has its advantages as it is relatively inexpensive and thus larger numbers can be deployed. Fruit is biodegradable and thus drogues that cannot be retrieved are less of a nuisance. However, in heavily contaminated areas where it is likely that the stranded fruit would be consumed by the resident population, then other –non-edible – objects may be considered. However, fruit can be more heavily influenced by winds and thus in some circumstances may not truly reflect the current speed and direction of the tide.

Often it is advantageous to use both winged and fruit drogues, with A winged drogue deployed at various depths, alongside fruit drogues. This can give a greater understanding of how much influence the wind may have at the surface. Using a large amount of fruit drogues may provide a greater sense of the overall dispersion or area that might be affected. In some situations where the current flow may split downstream into two channels, such as around a shoal, using a large amount of fruit deployed across the channel upstream (from one bank to the opposite bank) can help determine where in the channel the current flow paths may divide. It may also be advantageous to use different types of fruit (for example, oranges and grapefruits) to distinguish between batches that may be released at different times or locations. It is often advantageous to release drogues at various times within the tidal cycle to monitor changes in current speed and direction at different stages.

A7.3.3 *Batch releases of dye*

Batch releases of dye may be advantageous in areas where drogues may not be as feasible due to coastal features that might cause drogues to become stranded. Tracking can be done visually or by using a field fluorometer. Visual tracking would require use of more dye as additional batch releases may needed at a point when the dye is no longer visibly apparent.

Types of dye

There are different types of dye available for dye tracing. However, the use of a non-toxic dye should be used, such as Rhodamine WT, which has been approved for water quality tracing studies in the United States of America (Wilson *et al.*, 1986).

Amount of dye

The total amount of dye used will vary depending on the degree of tidal mixing and how often you may need to release a new batch of dye. Single one-litre batches of a dye+water mixture (50:50) are a good starting point. Several batches may be needed for an ebb or flood tide study. It is important to note that one might wish to release batches of dye at various times to determine changes in current speed and direction.

A7.4   USES AND ASSESSMENT OF DROGUE STUDIES

A7.4.1   *Time of travel*

Drogue studies are very useful in determining how long a pollutant, once released, takes to reach a location within the growing area. Determining the time of travel may be an important consideration in growing areas where the classification and management depends upon how quickly the authority can respond to a pollution event in order to close an area prior to its being affected by the source. In carrying out time of travel studies it is important to recognize that the current speed and direction may change during the course of the tide and several releases at different times may be beneficial. It is also important to consider additional factors as described above that may influence the results of the study.

A7.4.2   *Dispersion/dilution*

Understanding the dispersion of a pollution source under different conditions enables the determination of the overall area that may be affected by the pollution source. Using many drogues such as the fruit drogues or batch releases of dye may give a better sense of the dispersion of the pollutant as it travels through the estuary. A simple empirical plume dispersion calculation along with the time of travel data collected from the drogues may also be used as an estimate of the potential extent of plume dispersion and dilution over a limited time (refer to Annex 9).

A7.4.3   *Model calibration and validation*

Drogue studies are also useful for model calibration and validation of hydrodynamic models and can be used in conjunction with tidal and salinity data collected using a CTD (conductivity, temperature, depth) instrument. Whereas CTD data from sites at opposite ends of the study area may provide critical data on the amplitude of the tides and the changes of salinity from one location to the other, drogue data may also provide more relative data on the path the pollution source may travel. The time of travel and the transport patterns are important factors that should be considered in the calibration and validation of hydrodynamic models. Additional factors for consideration for hydrodynamic modelling are addressed in Annex 8.

REFERENCES CITED AND OTHER RESOURCES

Goblick, G.N. 2007. Advanced Growing Area Training – Conducting Hydrographic Studies, U.S. Food and Drug Administration. College Park, MD.

Wilson, J.F., Cobb, E.D. & Kilpatrick, F.A. 1986. Techniques of water-resources investigations of the United States Geological Survey: Fluorometric procedures for dye tracing. In: Application of Hydraulics, book 3, chapter A12. Washington, DC: United States of America. Geological Survey. Washington, DC: US Government Printing Office.

ANNEX 8

KEY CONSIDERATIONS IN UNDERTAKING AND ASSESSING HYDRODYNAMIC MODELLING

A8.1   POSSIBLE OBJECTIVES AND BENEFITS OF HYDRODYNAMIC MODELLING

> determine the time it may take for pollution sources to reach the growing area;

> determine the dispersion, dilution, and area affected by the pollution source;

> determine the build-up and residence time of the pollutant; and

> determine pollution source impacts under various conditions.

A8.2   DESCRIPTION OF INFORMATION AND DATA TO GATHER (IF AVAILABLE)

> *Bathymetry or Digital Elevation Model (DEM) data* – the elevation or the depth below surface of a tidal datum (e.g. Mean Sea Level). An essential component for hydrodynamic models that in conjunction with model boundary conditions largely dictates tidal flow conditions (direction, speed and amplitude);

> *Model boundary conditions* – the boundaries in a model where the input of flow is defined (tidal flow and freshwater inflow). Model boundary conditions can be derived based on data or generated through modelling;

> *Shoreline boundary data* – the boundary between the land and water relative to a tidal datum. Necessary for establishing model boundaries defined for computations;

> *Surface elevation and currents* – critical factors for model calibration. Typically, the tidal flow model calibration is performed by comparing model results of tidal water amplitude and period, with measured data;

> *Water column data (conductivity, temperature, depth measurements)* – useful for determining model selection (2D or 3D) and calibration based on water depths and stratification;

> *Pollution source* – volume rate of discharge (flow), discharge type (continuous or intermittent discharge) - may change due to daily water use patterns, hydrological events and seasonal influences. Also important are the pollution conditions (concentrations) which may change with changes in flow and level of treatment;

> *Navigation or any dredged channel locations* – defined channels may significantly influence tidal flow and thus in some circumstances additional channel data may be needed in addition to the bathymetry or DEM data. Structures or other large objects may also have an impact on tidal flow;

> *Wind* – history of prevailing wind direction and speed - may change with season. Particularly important in shallow estuaries with weak tides;

> *Freshwater inflow* – may change due to hydrological events and season. Particularly important when the relative contribution of freshwater is significant within the area modelled; and

> *Precipitation* – may apply where the freshwater inflow is a significant factor or when the flow rate or loading of the source may be influenced. In these cases, it is important to establish a relationship between the precipitation event and the relative contribution of flow or pollutant loading.

Note: Collection and preparation of data is one of the most time consuming steps of setting up a hydrodynamic model but it is one of the most important steps in order to produce results that are realistic and representative of the growing area and the typical range of conditions that could exist. As illustrated above, data requirements for hydrodynamic models can be significant. The specific type of information and data required typically depends on the complexity of the growing area, the type of model, and the objectives of the modelling study. Data availability in developing countries can be a limiting factor, thus data requirements and the availability of data should be considered in model selection. However, where data is limited the use of a complex model is still possible but may require the collection of additional field data. An excellent description of available models, data requirements and model capabilities can be found in Bahadur, Amstutz and Samuels, 2013.

A8.3   TYPES OF HYDRODYNAMIC MODELS

Hydrodynamic models may be described as one, two or three dimensional. One-dimensional models are limited to basic equations typically representing cross sectional averages in either the x-y (surface) or x-y-z (surface and depth) directions. Thus, one-dimensional models may not effectively model complex environments but rather a simpler environment such as a river flow that is predominantly well mixed and flows in one direction. Two-dimensional models represent variations in two dimensions usually in the x-y (surface) direction and may assume complete and uniform mixing in the z-direction (depth). Thus, two-dimensional models are more appropriate for growing areas that may have complex tidal current patterns but may have relatively shallow water depths or where stratification of fresh and sea water is

not a significant factor. Three-dimensional models represent variations in all three x-y-z directions and are more appropriate for growing areas where stratification or variation in pollution concentrations with depth are more significant.

A8.4   MODELLING AND SOFTWARE TOOLS, AND APPROXIMATE COST ($US) (VALUES AS OF LATE 2017)

> Commercial or open source hydrodynamic model software ($5 000 – $12 000+)

> Geographical Information System (GIS) software ($1 500–$6 000+)

In addition to hydrodynamic modelling software it is also beneficial to use GIS software to assist with processing data. Although open source modelling software may be free to use there could be a cost associated with compiling the data necessary to complete the model simulation. An advantage of open source software is that it may be less costly compared with commercial software and changes to the source code can be made by the user. A disadvantage with open source software is a potential lack of technical support and user friendliness in the overall appearance and operation of the model compared with commercial products. Additional commercial software can be purchased to facilitate and enhance DEM data processing. The resolution of DEM data is a very important component for setting up a model. It can be obtained from open sources and modified in ArcGIS for modelling use. However, if needed, higher resolution DEM data may also be purchased commercially.

A8.5   CONDUCTING A HYDRODYNAMIC MODELLING STUDY

A8.5.1   *Factors to consider*

Tidal depth and stratification

Please refer to Annexes 7 and 9 regarding factors to consider with respect to tidal depth and stratification. Additional factors to consider at locations where there are no buoy locations that can provide tidal information are that collection of measured data may be necessary. A conductivity, temperature and depth (CTD)-type instrument may need to be deployed in the study area for at least two weeks, covering both spring and neap tides. Accurate tidal data is an essential first step in getting a good calibration of tidal flow. CTD measurements of the water column (measuring from the surface to the bottom depths) may be useful in determining if waters are well mixed or show a large gradient in density and are thus considered stratified. Highly stratified waters typically indicate that a pollutant will be most unlikely to be evenly mixed with depth. CTDs positioned on each end of the study area can provide useful information regarding the potential lag time in surface elevation (useful for calibration). Also, salinity measurements between the two locations and salinity tows (measuring salinity starting from freshwater source into more saline waters) may be useful for validating the pollutant transport model.

Wind

Please refer to Annexes 7 and 9 regarding factors to consider with respect to wind. Additional factors to consider – wind may not only influence the degree of mixing within the water column but may also affect both the transport speed and direction of a pollutant. This may be more apparent in estuaries that are more open to the wind and where the fetch length, the length of water over which wind can travel unimpeded, is great. Wind may be the most dominant factor in shallow estuaries with weak (mesotidal) tides. In these situations, wind may be the most important factor in producing realistic model simulation results. A hydrodynamic model sensitivity analysis may be useful to gauge the significance of wind on the mixing and transport of a pollution source in a growing area. If the result of varying winds within the ranges expected are significant then more attention may be needed to accurately reflect the wind conditions.

Freshwater inflow

Please refer to Annexes 7 and 9 regarding factors to consider with respect to freshwater inflow. A hydrodynamic model sensitivity analysis may be useful to gauge the significance of freshwater flows on the travel time, dilution, and transport of a pollution source within a growing area. If the result of varying freshwater flows is significant to the results from the model, then more attention may be needed to accurately reflect the freshwater inflow conditions.

Bathymetry, navigation channels and structures

Please refer to Annex 7 regarding factors to consider with respect to tidal depth, bathymetry, navigation channels or structures. Additional factors to consider – higher resolution bathymetry or DEM data, produce more accurate (speed and direction) tidal flow modelling results. DEM data is described in more detail by Lim *et al.* (2009). Dredged channels can be of particular importance, which might not be reflected accurately on bathymetry charts, depending on when the data was collected and the charts produced. It may be important to obtain data for dredged channels from the authority responsible for dredging if channels are not accurately represented with the bathymetry data. Measured data can be used to adjust the computational element mesh (width and depths) associated with the channel.

Pollution source

Please refer to Annex 3 for a checklist intended to assist in determining whether a treatment works is functioning correctly. It is important to conduct an assessment of the pollution source prior to planning a dye study. The flow rate and the concentration of the pollutant is critical in simulating the total load discharged over a specified time. If the modelling study is to assess a treatment works, various flow rates may need to be considered. For example, if a treatment works has a combined collection system which collects sanitary waste as well as storm water then the flow

rate of the treatment works may be highly variable particularly during periods of wet weather. A treatment works that has a separated collection system and treats primarily sanitary waste may be less influenced by wet weather. However, the age of the collection system can be a factor and even a separated system can experience a significant increase by inflow and infiltration into a leaky collection system from groundwater and stormwater. Modelling studies can also be conducted to assess the impact of polluted tributaries on the shellfish growing area. Often, the pollutant loading (which is calculated as flow rate of a pollutant times its flow rate) may be greatest under wet weather conditions, which may increase the tributary flow and often the pollutant concentration. When simulating a storm-related event it is often best to begin the simulation prior to the event during "baseline" conditions, and running the simulation until the growing area recovers and returns to the baseline conditions, to determine the duration of impact.

A8.6   TYPICAL MODELLING PROCESS

Typically, hydrodynamic models are split into two components – a flow model and a transport model. The flow model simulates the hydrodynamic conditions such changes in water elevation and current speed and direction. The flow model results are then used in the transport model which simulates the fate and transport of pollutants in the growing area. In some cases, both the hydrodynamic and the transport models are integrated into a single model.

The typical steps necessary to produce a model are:

> *Model set-up* – Gathering and processing data for setting up the model, then calibrating and validating. The gathering and processing of data for input and comparison is often the most time consuming step. During the model set-up computational mesh elements are established, typically extending beyond the study area of interest and out of the influence of the model boundary conditions. An example of a hydrodynamic computational mesh is shown in Figure A8.1. Boundary conditions drive the circulation of a hydrodynamic model. Usually, a time-series of water surface elevation at the boundary needs to be specified based on gauge data collected or generated from the model.

> *Model calibration* – Adjusting modelling parameters within expected ranges to match measured data as well as possible. A sensitivity analysis can be performed to determine which parameters have the most influence on model results to determine where measured data is most needed. Once the mesh and boundary conditions are set up, the model can be calibrated to produce accurate predictions of tidal flow. Where buoy data within close proximity to the modelled area is not available CTD data placed at opposite ends of the study area may provide critical data on the amplitude of the tides and the changes of salinity from one location to the other. Often, the modelled tidal elevations may be calibrated against the

measured tidal elevation data, and salinity data is often used to validate the change in concentration of a conservative pollutant. A comprehensive manual addressing model calibration and quality control is provided by Bartlet (1998). An example of a hydrodynamic model calibration of generated tidal elevation and period against measured CTD is shown in Figure A8.2.

> *Model validation* – Testing the calibrated model against a second, independent, data set, ideally under different conditions, to verify the model's ability to reasonably represent the shellfish growing area under various hydrodynamic and pollutant loading conditions. In addition to salinity data mentioned above, dye studies are also useful for model validation and may provide more relative data on the path the pollution source may travel. The time of travel and the transport patterns are critical factors that can be preferably validated through dye studies or, at a minimum, drogue studies. Dye studies can also provide important information regarding the build-up of pollutants and the overall residence time and can be used to compare with model results. Drogue and dye studies are discussed in more detail in Annexes 7 and 9, respectively.

> *Model application* – Model various scenarios to determine or verify the shellfish growing area classification and various management strategies that could be employed. The following section addresses types of model simulations to address various growing area classification objectives.

A8.7   USES AND ASSESSMENT OF HYDRODYNAMIC MODEL STUDIES

A8.6.1   *Model simulations to address objectives*

Short-term event (failure)

The duration of the simulation should match the duration of the expected response time needed to close and enforce the growing area after a sewage release event. The model simulation should be long enough in time to determine the overall maximum extent affected over several tidal cycles and to determine the time needed for the shellfish growing area to recover to background levels. Modelling a failure event occurring under various tidal conditions and potential pollutant loadings may be useful to establish worst-case conditions.

Longer-term event (failure) or continuous release pollutants

For pollutants that may be released for long durations (several tidal cycles) or discharge continuously, the simulation can also be conducted with a continuous release of pollutants (simulating days or weeks) until a steady state maximum is achieved. Modelling various tidal conditions and potential pollutant loadings may be useful to establish worst-case conditions.

Overboard discharge or batch releases of pollutants

For discharges that are not continuous in nature, such as a treatment works that may release sewage on outgoing tides, modelling can be conducted for simulating this type of intermittent or batch sewage release. For the intermittent type of discharges the frequency and duration can be assessed under different tidal conditions to determine the worst case. Batch release simulations are also useful for assessing the impact of an overboard discharge from boating activities or other activities (such as fish farms), where discharges could potentially take place. It is important to recognize that the current speed and direction may change during the course of the tide and several batch release simulations at different times and tidal conditions may be beneficial in determining worst case.

FIGURE A8.1 **COMPUTATIONAL MESH ELEMENTS WITH BATHYMETRY DATA**
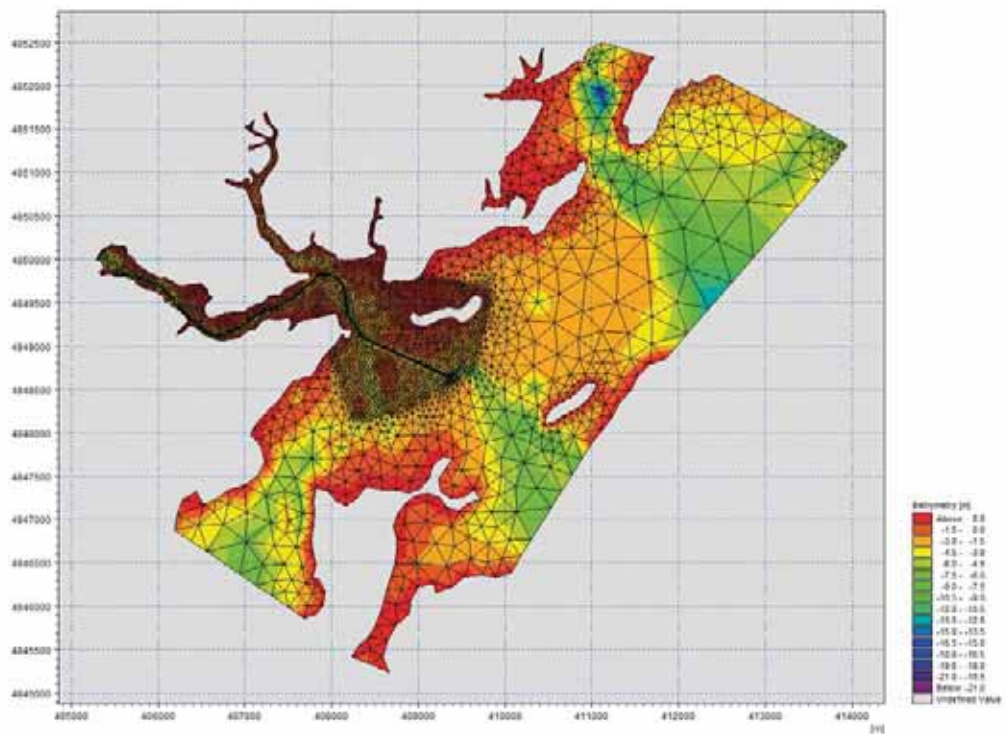
FIGURE A8.2 **MODEL CALIBRATION OF TIDAL SURFACE ELEVATION – CTD FIELD MEASUREMENT (DOTTED LINE; RED) VS. MODEL SIMULATION (CONTINUOUS LINE; BLUE)**
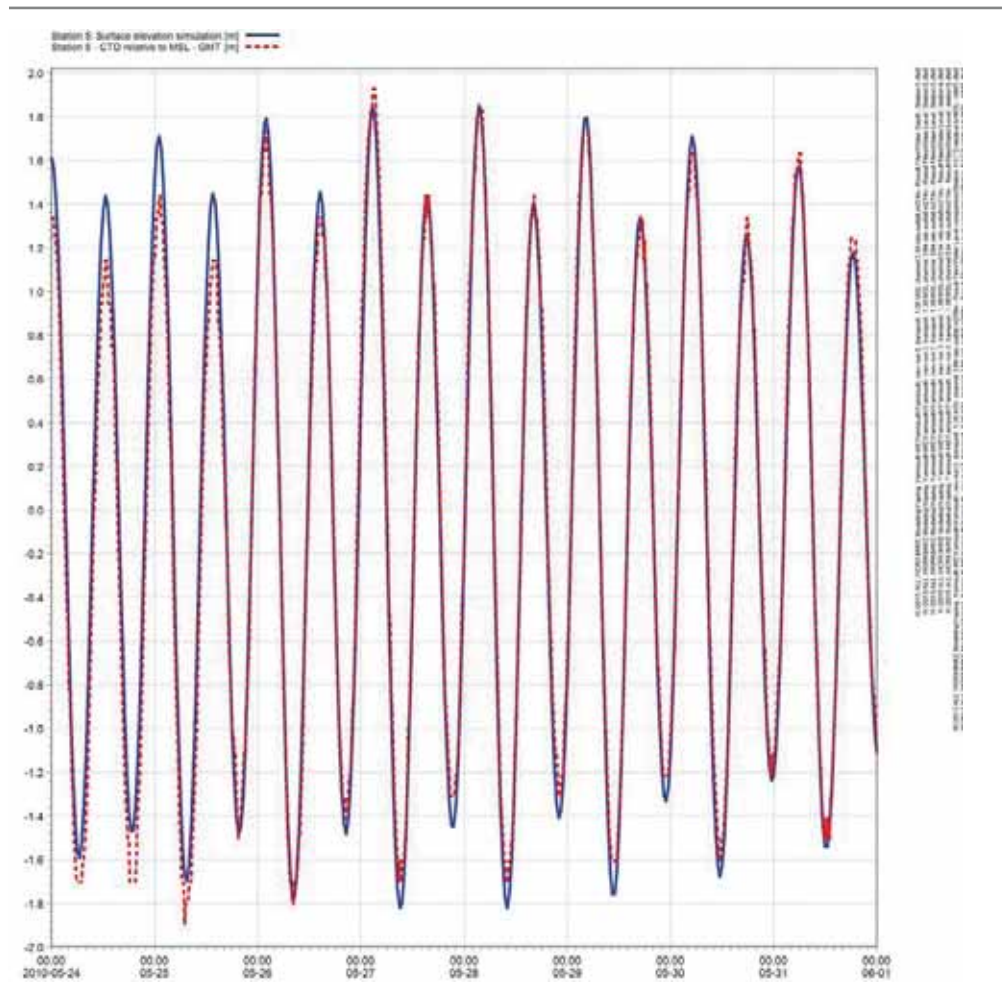
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Lim, E., Taylor, L.A., Evans, B.W., Carigan, K.S., Warnken, R.R. & Medley P.R. 2009. Digital Elevation Model of Portland, Maine: Procedures, Data Sources and Analysis, NOAA Technical Memorandum NESDIS NGDC-30, National Geophysical Data Center, Boulder, CO.

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ANNEX 9

KEY CONSIDERATIONS IN UNDERTAKING AND ASSESSING A DYE STUDY

A9.1   POSSIBLE OBJECTIVES AND BENEFITS OF THE STUDY

> Determine the time it may take for pollution sources to reach the growing area;

> determine the dispersion, dilution and area affected by the pollution source;

> determine the build-up and residence time of the pollutant; and

> Provide results that can be used to calibrate and validate model results.

A9.2   INFORMATION TO GATHER (IF AVAILABLE)

> Bathymetry data for the water body;

> navigation or any dredged channel locations;

> wind – history of prevailing wind direction and speed (Note – may change with season);

> freshwater inflow – may change due to hydrological events and season;

> structures or other objects that may be useful for deploying moored equipment;

> tidal flow – water level and currents;

> pollution source – volume rate of discharge (flow), discharge type (continuous or intermittent discharge). These may change due to daily water use patterns, hydrological events and seasonal influences.

A9.3   NECESSARY MATERIALS AND EQUIPMENT; AND APPROXIMATE COST (US DOLLARS; AS OF MID-2016)

> Submersible fluorometer for tracking dye from vessel ($5 000);

> Submersible fluorometer for moored station ($8 000);

> Peristaltic dye injection pump ($ 900);

> Dye injection tubing ($ 75/10 metres);

> Rhodamine WT 20 percent liquid concentrate dye ($ 50/litre); and

> Field computer with integrated Global Positioning System (GPS) ($ 2 000).

The above materials and equipment are the minimum recommended to conduct a hydrographic dye study. The submersible fluorometer for tracking dye from a vessel (towed behind a boat) allows for collecting data on the time of travel and the spatial aspect of the plume dispersion and area of impact. Moored submersible fluorometers can capture data at programmed intervals for several days including times when collecting data from a vessel may not be feasible (e.g. at night or in foul weather). Additional equipment may also facilitate and enhance data collection, such as a Conductivity, Temperature, Depth (CTD) unit with integrated fluorometer that is capable of surveying profiles (collecting measurements at various depths). Additional submersible fluorometers used for moored stations and allow a greater coverage throughout the growing area.

A9.4   FACTORS TO CONSIDER

> *Tides:* Please refer to Annex 5 regarding factors to consider with respect to tides.

Additional tidal factors to consider relate to the timing of the dye release. In general, as tides rise due to the gravitational force of the Moon and Sun acting on the ocean's water, there will be a current flowing from the ocean to the estuary, which is typically referred to as a "flood" current. An "ebb" current occurs when the tides fall and a current flows from estuaries towards the oceans. "Slack" currents are defined as period when there is little to no movement of water. However, it is important to note that the times of high and low water do not always coincide with the times of slack currents, and the relationship between tides and tidal currents is unique to each location. Therefore, it is often best to conduct a preliminary drogue release before the release of dye to determine more accurately the period of slack currents for the given study area and within the vicinity of the intended dye release. Another tidal characteristic that needs to be considered is the period of the tide. Tides are typically semi-diurnal (two high water and two low waters each tidal day) or diurnal (one tidal cycle per tidal day) in nature. Tidal constituents including the position of the Moon and Sun relative to the earth, the altitude of the Moon, the Earth's rotation and bathymetry, all these may influence the characteristics of the tide. As a result, for semi-diurnal tides the heights of the two high waters and low waters may not be the same. When there are two high tides and two low tides each tidal day, and the difference in heights are more extreme, the pattern is typically referred to as a mixed semi-diurnal tide. The tidal pattern for the given location may influence the duration of the dye injection, as discussed further below.

Wind

Please refer to Annex 5 regarding factors to consider with respect to wind. Additional factors to consider include that wind may influence the degree of mixing within the water column. For example, dye-tagged pollution sources that are buoyant in nature and rise to and transport near the surface may become further mixed within the water column by wind action. This may be more apparent in estuaries that are more open to the wind and where the fetch length (the length of open water over which wind may travel) is great.

Freshwater inflow

Please refer to Annex 5 regarding factors to consider with respect to freshwater inflow. Additional factors to consider include storm events that occur during the study period, as they may potentially affect the level of background fluorescence. Thus, proper consideration must be given to establishing the background fluorescence level before study, and also during the study, in areas outside the influence of the dye tag pollution source, to ensure background levels are properly determined. Accurate background measurements allow for more accurate dye study results, with less chance for false positives caused by improperly establishing the level of background.

Tidal depth and stratification

Please refer to Annex 5 regarding factors to consider with respect to tidal depth and stratification. Additional factors to consider are that study areas with deeper waters may require specialized instruments that can measure dye concentrations at a greater depth than just below the surface. This may especially be needed in situations where there is a large depth over the discharge source, which may increase the chance that the plume may stay trapped below the surface for an extended duration or distance from the discharge location.

Bathymetry, navigation channels and structures

Please refer to Annex 5 regarding factors to consider with respect to tidal depth bathymetry, navigation channels and structures. Important consideration needs to be given to siting if instruments to collect data will be moored within the study area. Instruments should be placed in areas that are out of the way of boat traffic. It is also important to consider tidal depths and currents, avoiding high current areas or areas that under some tidal conditions become too shallow, leaving instruments exposed. Placing instruments out of shipping channels and just on the outside of channel markers, buoys or other structures, helps to protect instruments from being run over by boats, and aids when retrieving the instruments.

Pollution source

Please refer to Annex 3 for a checklist intended to assist in determining whether a treatment works is functioning correctly. It is important to conduct an assessment of

the pollution source prior to planning a dye study. The flow rate of the pollution source is critical in determining the total amount of dye that will need to be injected over a specified time and a flow rate of the dye feed that will be needed. If the dye study is to assess a treatment works, various flow rates might need to be considered. For example, if a treatment works has a combined collection system that collects sanitary waste as well as storm water, then the flow rate of the treatment works may be highly variable, particularly during periods of wet weather. A treatment works that has a separated collection system and treats primarily sanitary waste may be less influenced by wet weather. However, the age of the collection system can be a factor and even separated system can experience a significant increase through inflow and infiltration into a leaky collection system through groundwater and storm water. Being prepared in advance for the potential range of flows that might occur during the study period allows for last minute adjustments to be made more easily to account for the given conditions. Dye studies can also be conducted to assess the impact of polluted tributaries on the shellfish growing area. Often, the pollutant loading (which is calculated as flow rate of a pollutant times its flow rate) may be greatest in wet weather, and that may increase the tributary flow, and often also the pollutant concentration. If possible, targeting worse-case periods in terms of pollutant loadings is preferred.

A9.5   TYPES OF DYE RELEASE

Single tide release

A single-tide release that occurs over only an ebb or flood tide may be useful for simulating the impact of a short-term sewage release. For example, if it is determined (through an assessment of the treatment works and enforcement capabilities) that a growing area can be closed and enforced within <6 hours after a sewage release event (for a semi-diurnal tide) or <12 hours (for a diurnal tide) then it may be possible to simulate this event through a dye release on a single tide. To determine the maximum extent of the area of impact, the dye release would typically occur at the beginning of the ebb or flood tide and be tracked through the duration of the tide. The duration of the injection should match the duration of the expected response time needed to close and enforce the growing area after a sewage release event. Additional tracking may need to be conducted on subsequent tides to determine the overall maximum area affected.

One-half tidal day release

For simulating a sewage release of longer duration or the pollutant impact from a continuously discharging source, a one-half tidal cycle dye release may be conducted for semi-diurnal tides. The cycle (e.g. flood-ebb) is completed in one-half of a tidal day for a semidiurnal tide with a tidal period of 12.42 hours (based on a mean one-half lunar day). Injecting dye into a pollutant source for a complete cycle enables the estimation of the overall build-up of pollutants and a maximum concentration of a pollutant at steady state that may occur after several tidal cycles. With this method, referred to as "super position", determinations are achieved by cumulative measurements taken on several tidal days and superimposing in cumulative fashion the measurements

taken on each tidal day after the dye injection, on to the measurements recorded on the first tidal day. This process is continued until a stable (peak) concentration value is obtained. The peak concentration value represents the build-up of pollutants to a steady state maximum, and the period to reach that steady state represents the overall residence time of pollutants within the estuary. This method is described in more detail in Goblick *et al.*, 2011, and Kilpatrick and Cobb, 1985.

Whole tidal day release

For simulating a sewage release of longer duration or the pollutant impact from a continuously discharging source, a whole tidal-cycle dye release may be conducted for diurnal tides. The cycle (flood–ebb) is completed in a whole tidal day for a diurnal tide with a tidal period of 24.84 hours (based on a mean lunar day). A whole tidal day release may also be necessary for a mixed semi-diurnal tide when there are two high tides and two low tides each tidal day that have an extreme difference in heights. Similar to the one-half tidal cycle method of super position, the degree of pollutant build-up, the steady state maximum concentrations, and the overall residence time of pollutants can be determined.

Batch release

Batch releases of dye may be useful in many different situations. Batch releases of dye may be used for discharges that are not continuous in nature, such as a treatment works that may release sewage on outgoing tides. For an intermittent type of discharge the frequency and duration should be assessed and compared with the tidal conditions representing a worst case, when selecting the timing of the dye release. A batch release of dye may also be necessary when assessing a treatment works with a very large flow rate, when the cost of dye to conduct a release over a longer duration is not feasible. A batch release conducted under the right conditions for a high-flow treatment works can still be useful in determining the time of travel from the point of release to the growing area and the initial dilution on a single tide within a 1 000:1 dilution. Batch releases are also useful for assessing the impact of an overboard discharge from boating activities or other activities (such as fish farms) where discharges could potentially take place. It is important to recognize that the current speed and direction may change during the course of the tide and several batch releases at different times may be beneficial.

A9.6   TYPE AND AMOUNT OF DYE

The majority of field fluorometers used for measuring dye concentrations are configured for the use of Rhodamine WT dye. Rhodamine WT is a bright fluorescent red dye typically purchased as a liquid concentrate with 20 percent dye content. Rhodamine WT is considered a non-toxic dye and has been approved in the United States of America for water quality tracing studies by the U.S. EPA. The total amount of dye used will vary depending on the type and duration of the dye release,

the flow rate of the pollutant source, background fluorescence measured prior to the dye release, and limit of detection of the instruments. Most importantly, the amount of dye used is also dependent on the objective of the study. For example, if the objective of the study is to determine the size of a prohibited zone that is large enough to dilute the pollutant loading of a treatment works malfunction then a sufficient amount of dye needs to be used to be able measure a high level of dilution (e.g. >50 000:1 to100 000:1 dilution) in the growing area. However, if the goal is to establish a minimum size of prohibited zone based on the treatment works operation under normal operating conditions (e.g. for establishing a conditional area based on the efficiency of the treatment works and closing the conditional area when the treatment works malfunctions), then a lesser amount of dye may be used to be able to measure a lower level of dilution (e.g. 1 000:1). Thus, it is important to establish the level of dilution needed to meet the intended type of classification and management strategy.

A9.7 MODEL CALIBRATION AND VALIDATION

Parameters for carrying out the study:

> Dye studies are also useful for model calibration and validation of hydrodynamic models and can be used in conjunction with tidal and salinity data collected using a CTD instrument. CTD data from sites at opposite ends of the study area may provide critical data on the amplitude of the tides and the changes in salinity from one location to the other.

> Dye study data may, however, provide more relative data on the path the pollution source may travel.

> The time of travel and the transport patterns are also critical factors that should be considered in the calibration and validation of hydrodynamic models.

> Dye studies can also provide important information regarding the build-up of pollutants and the overall residence time, and can be used to compare with model results. Additional factors for consideration for hydrodynamic modelling are addressed in Annex 6.

A9.8 REFERENCES CITED

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Kilpatrick, F. A. &Cobb, E.D. 1985. Techniques of water-resources investigations of the United States Geological Survey: measurement of discharge using tracers. pp. 1–52, *in: Application of Hydraulics*, book 3. U.S. Geological Survey report no. TWRI3A16. Washington, DC: US Government Printing Office.

ANNEX 10

BUFFER ZONE DETERMINATION WITH RESPECT TO ENTERIC VIRUSES

A10.1 INTRODUCTION

Establishment of buffer zones around inputs of human sewage pollution is an integral component of an effective bivalve mollusc sanitation program. An area classified as prohibited for bivalve mollusc harvesting for human consumption is normally established adjacent to sewage sources such as sewage treatment works (STWs) outfalls, lift (pumping) stations, combined sewer overflows, marinas, aquaculture operations, or any source of sewage pollution that may potentially have a bearing on the sanitary quality of the harvest area. The designation of prohibited buffer zones is a preventative public health measure principally aimed at protection against contamination of bivalve molluscs with human enteric viruses. The classified growing area should meet both the relevant buffer zone requirement and the microbiological compliance criteria for the relevant category.

The approach to buffer zones given in this annex can also be applied to watercourses carrying sewage effluent (treating the mouth of the watercourse as a point source of diluted sewage) as well as other activities which may pose a risk of introducing enteric viruses in the growing area such as marinas and fish farms, etc. The principle of buffer zones can also be applied to other types of hazard associated with point sources of contamination, such as many chemical contaminants and radionuclides. While many of the concepts given in this annex apply, buffer zones for these other hazards will not be specifically considered here. At present commercial short-term relay and depuration (purification) processes have not been shown to significantly reduce the concentration of enteric viruses in bivalve molluscs, the same approach to the determination of buffer zones has been recommended for Category I and Category II areas.

A10.2 ESTABLISHING PROHIBITED BUFFERS ZONES FOR SEWAGE DISCHARGES

During usual STW operation (treatment with a combination of physical, chemical, and biological processes to remove contaminants), sewage treatment that includes disinfection[6] has been shown to be effective in reducing bacteria to levels well below bivalve mollusc growing area standards. However, human enteric viral pathogens such as norovirus (NoV) and hepatitis A virus (HAV) are more resistant to disinfection and are not reduced to the same degree as bacterial indicators (Burkhardt *et al.*, 2005). Thus, the reduction of indicator bacteria in effluent after disinfection eliminates the effectiveness of routine bacterial monitoring in determining the safety of bivalve molluscs with respect to enteric viruses and cannot be used to establish prohibited buffer zones adjacent to STWs. Additionally, although treatment technologies have improved, STWs are still subject to failure regardless of the type of treatment system used. Therefore, when sizing a prohibited buffer zone, consideration should be given to the performance of the STW including situations of failure or degraded treatment and it is highly recommended that prohibited buffer zones should be sized according to the most likely type of worst case failure/degraded treatment event that could occur. A significant factor in determining the size of the buffer zone and the management of the growing area is the classification of the adjacent growing areas to the STW discharge. The following section discusses some of the possible classification scenarios adjacent to STWs.

A10.2.1 *Classification of adjacent waters in relation to discharge location*

For sewage discharges subject to no treatment, or to primary settlement only, it is assumed that there is no effective reduction of enteric viruses prior to discharge and the sizing of a buffer zone is based on the microbiological loading of the influent. For discharges subjected to secondary treatment or disinfection, the most conservative approach in managing a growing area in proximity to a STW would be sizing of a buffer zone in consideration of a failure in treatment. This would enable the adjacent bivalve mollusc growing area (Category I, II or III) to remain open under any operating condition at the STW including a failure in treatment. In this regard, the performance of the STW under normal operation is of less concern than the operation under a failure event. Although establishing a buffer zone based on failure condition may prevent harvesting a large area adjacent to the STW this approach requires markedly less resource than does the control of a conditionally managed area. There are many potential classification scenarios STWs but the following examples are the more common.

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[6] Reference to disinfection in this annex should be taken to include other types of tertiary treatment, intended to reduce the concentration of pathogens in sewage prior to discharge. However, some types of ultrafiltration are effective in the removal of viruses when operated optimally.

Scenario 1: Buffer zone sized for an untreated discharge or failure adjacent to a Category I or II area

The following example demonstrates a prohibited zone that is sized based on a STW failure and is adjacent to a Category I or II area as shown in Figure 1.

FIGURE A10.1   **BUFFER ZONE SIZED FOR FAILURE ADJACENT TO A BIVALVE MOLLUSC GROWING AREA**
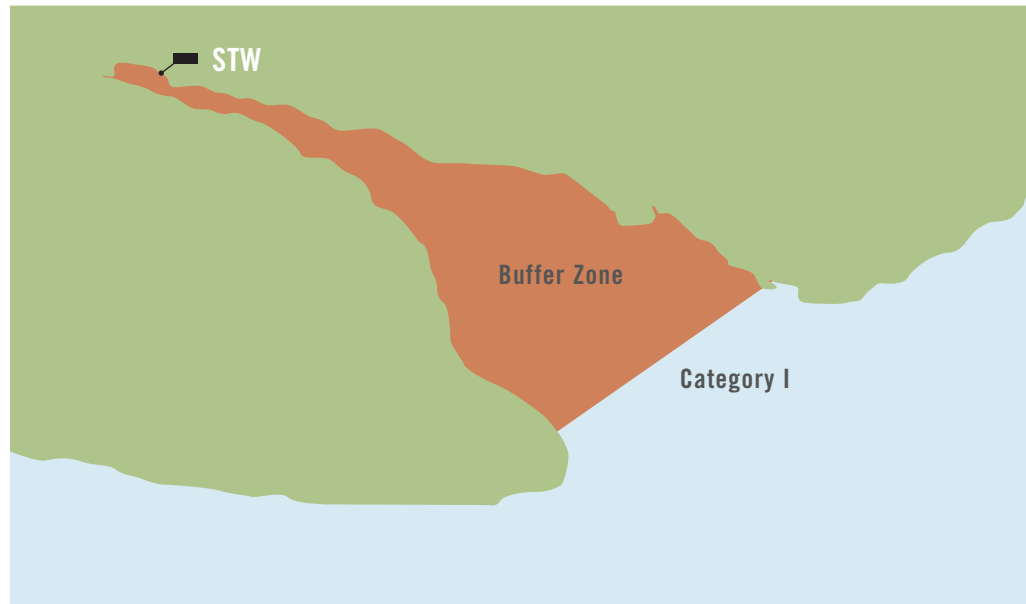
In the above scenario, regardless of the intended treatment level, and actual performance of the STW, the prohibited zone is sufficiently sized such that the classification of Category I (or Category II, as relevant) is met under all STW and growing area conditions including a failure at the STW. For this type of classification, it is recommended that a minimum dilution is met at all points within the prohibited zone under all conditions in consideration of a raw sewage release based on reducing microbiological indicators to the relevant classification criteria. Recommended dilution values are provided in part b below as a point of reference. An example dilution calculation assuming a STW failure is provided in Section 4 (dilution analysis) of this annex.

Scenario 2: Minimum sized buffer zone based on conditional management and performance of STW

With the advancement of STW technologies, such as improved monitoring and alarm systems, it may be possible to safely operate and manage a conditionally approved bivalve mollusc growing area based on the operation of the STW. The definitive management condition in such a circumstance is the operational effectiveness of the STW to remove or inactivate viral pathogens. In some situations, alarm and

notification procedures for growing area management are so effective that notification times may be reduced to several hours. Accounting for both adequate dilution and time of travel are critical to producing safe bivalve molluscs wherever conditionally approved areas are adjacent to prohibited areas established for STW discharges.

For example, conditional management could be an option to potentially maximize the size of a Category I areas adjacent to **highly efficient** and **well monitored** STWs as shown in Figure 2. Under conditional management, it may be possible to operate part of the growing area at a better classification (Category I or Category II) when the STW is operating within defined **normal** operating parameters. Under such conditions there would still be a need for a minimum sized buffer zone based on the performance of the STW. However, under conditions **outside of normal** operation the conditionally managed part of the growing area would need to close in a timely manner[7].

FIGURE A10.2 **OPERATION OF A CONDITIONALLY MANAGED AREA DURING "NORMAL" STW OPERATION**
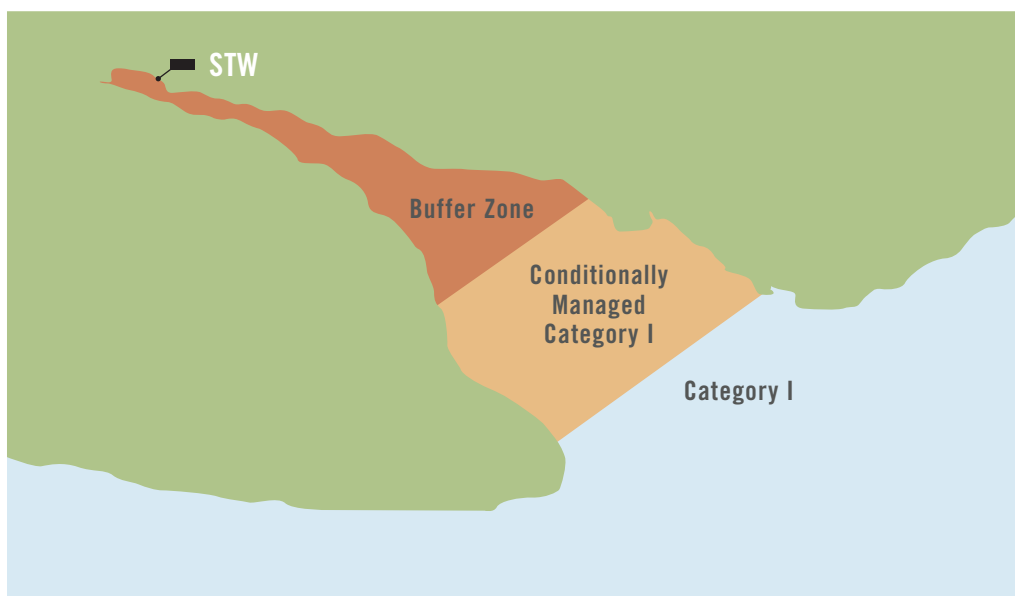
It should be cautioned that operating a conditionally managed area is also highly dependent on the resources and ability of competent authorities to monitor and enforce the growing area during a failure condition. Thus, conditional management should only be considered for STWs that are given a proper assessment (see the STW Questionnaire in Annex 3). It is also highly recommended that the efficiency of the STW is validated through MSC sampling as discussed further in parts b-c of this annex and in Annex 11.

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[7] The responsible authority should be able to notify, and enforce, closure of the conditionally managed part of the growing area before the additional pollution reaches the boundary of the growing area closest to the discharge.

Scenario 3 – Reduced buffer zone - utilizing long-term relay or other appropriate post-harvest treatment

To allow harvesting closer to the STW discharge than would be possible under Scenario 1, and without the need to meet the requirements for conditional management, a Category IIIa or IIIb[8] area could be operated (provided the classification criteria are met) and the harvested product relayed to a Category I area for long-term relay (Category IIIa) or subject to other appropriate post-harvest treatment (Category IIIb) to address the viral risk posed by the discharge from the STW. In this scenario, illustrated in Figure 3, a minimum dilution should be met at all times between the prohibited buffer zone and the Category III area and the minimum dilution between the prohibited zone and the Category III area should be outside of any established regulated mixing zone[9] that initial effluent dilution occurs or any areas where acutely toxic conditions may be present.

FIGURE A10.3 **BUFFER ZONE ADJACENT TO A CATEGORY III AREA**
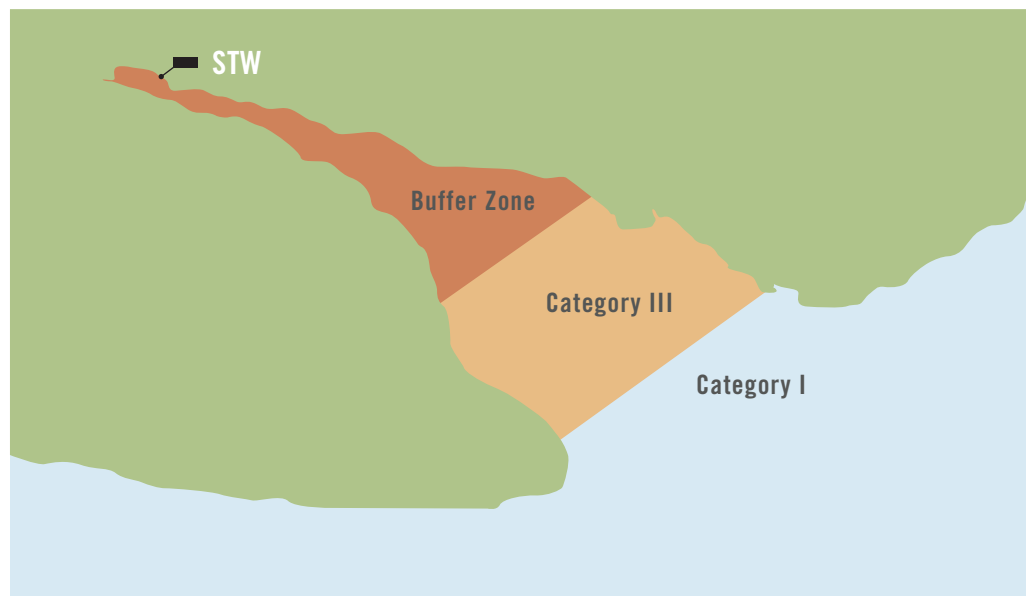
The duration of time needed to reduce viruses present in bivalve molluscs harvested from Category III areas relayed to a Category I is dependent on many factors including the performance of the STW, the minimum dilution provided by the

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8   If the hazard(s) for which the area has been designated as Category IIIb is(are) relevant to the point source of pollution. For example, this would not be the case for areas classified as IIIb based on the risk from naturally occurring vibrios.

9   Mixing zones are areas where an effluent undergoes initial dilution and are extended to cover secondary mixing in the water body. Mixing is affected by the momentum and buoyancy of the discharge and the motion and turbulence of the water receiving the discharge. A regulated mixing zone is one defined by the environmental regulator for the purposes of environmental water quality regulation.

prohibited zone, as well as species of bivalve mollusc and environmental conditions in the growing area. Annex 11 provides information on the use of MSC in the verification of long-term relay for the removal of enteric viruses from bivalve taken from Category III areas.

Additionally, if the Category III area is adjacent to a Category I or II area, there should also be a minimum dilution within the Category III area such that the conditions of the adjacent area are met at the boundary (e.g. such as the boundary between the Category III and I area as shown in Figure 3). This minimum dilution should be consistent with Scenario I above where the amount of dilution necessary is based on a raw sewage release or the most likely type of worst case failure/degraded treatment event that could occur. Recommended minimum dilution ratios based on STW efficiency benchmarks are provided in part b below. A brief explanation of the basis for the recommended minimum dilution values is presented in Annex A10.1.

When operating a Category III area adjacent to a STW the minimum buffer zone should be equal to or greater than any regulated mixing zone or near-field mixing zone where initial effluent dilution occurs and where toxic conditions may exist. In the absence of a regulated mixing zone or understanding of the near field mixing zone the minimum dilution ratios presented below and as shown in Table 1 may also be used when sizing buffer zones adjacent to Category III areas.

Characterizing STW performance – recommended benchmarks

In consideration of bivalve mollusc growing areas, the performance of the STW can be separated into the following benchmarks (with respect to bivalve mollusc classification only) as indicated below:

Minimum Dilution Standard of =>300:1 normal operation (with management plan)

> STWs are capable of meeting the following criteria:

> Consistently achieve >3 log reduction of MSC (90th percentile)

Minimum Dilution Standard of =>1 000:1 normal operation (with management plan)

> STWs are capable of meeting the following criteria:

> Consistently achieve >2.5 log reduction of MSC (90th percentile)

Minimum Dilution Standard of =>10 000:1 normal operation (with management plan)

> STWs are capable of meeting the following criteria:

> Consistently achieve >1.5 log reduction of MSC (90th percentile)

Minimum Dilution Standard of =>100 000:1 or =>350 000:1

> This is the minimum dilution that should be used for sizing the prohibited zone if:

> The responsible authority chooses not to manage a growing a conditionally based on the performance of the STW and where no relevant data exists to support a lower dilution threshold.

> A STW efficiency assessment is conducted and the STW fails to meet the above criteria.

> The >100 000:1 is based on the determination that the worst-case failure situation is a partially treated sewage event (no history of complete bypass in treatment where no treatment occurs).

> The > 350 000:1 is based on the determination that the worst-case failure situation is a raw sewage event (no treatment occurs).

Additional factors that should be considered in the classification of a STW are as follows:

> Time of travel/response time to a STW malfunction/operating outside of normal.

> History of raw sewage discharges, bypasses, or lapses in any stage of treatment including disinfection.

> Capacity to treat the typical range of flows entering the STW relative to the design capacity of the works.

> Monitoring capability including staffing and automated alarm systems such as supervisory control and data acquisition (SCADA) monitoring.

A lower $\log_{10}$ reduction value indicates fewer viruses are removed or inactivated by the STW, whereas a higher $\log_{10}$ reduction value indicates that more viruses are removed or inactivated. The geometric mean is a type of mean used to determine the central tendency of efficiency of the works represented as a log reduction. The 90th percentile of the log reduction values gives in indication of the range of performance including a period when the log reduction is lowest and the performance of the STW is poor. Thus, the 90th percentile to indicate a lower limit of log10 reduction considered as in a range of normal/efficient operation should be used when establishing the overall performance of the STW. Table A10.1 summarizes target benchmarks provided above for determining STW efficiency and the corresponding minimum dilution.

TABLE A10.1 **DILUTION RATIO BENCHMARKS ADJACENT TO SEWAGE DISCHARGES**

STW BENCHMARK MSC 90%TILE LOG REDUCTION	MINIMUM DILUTION FOR BUFFER ZONE – NORMAL STW OPERATION	MINIMUM DILUTION FOR BUFFER ZONE – PARTIALLY TREATED OR NO TREATMENT	COMMENTS
>3 log	>300	>100 000 or >350 000	For conditional management 300:1 (or determined) minimum size otherwise size for partial or no treatment

STW BENCHMARK MSC 90%TILE LOG REDUCTION	MINIMUM DILUTION FOR BUFFER ZONE – NORMAL STW OPERATION	MINIMUM DILUTION FOR BUFFER ZONE – PARTIALLY TREATED OR NO TREATMENT	COMMENTS
>2.5 log	>1 000	>100 000 or >350 000	For conditional management 1 000:1 (or determined) minimum size otherwise size for partial or no treatment
>1.5 log	>10 000	>100 000 or >350 000	For conditional management 10 000:1 minimum (or determined) size otherwise size partial or no treatment
Partially treated <1.5 logs	NA	>100 000	For partially treated sewage use minimum 100 000:1 (or determined)
Raw/influent	NA	>350 000	For raw sewage (no treatment) use minimum 350 000:1 (or determined)

Based on the above recommended benchmarks, conditional management should be used for dilutions less than 100 000:1 or 350 000:1 (which are based on the most likely failure to occur at the STW). If the competent authority does not wish to undertake conditional management due to the resources needed determine the performance of the STW and responding in a timely manner to periods of degraded treatment and failure (requiring timely closure of the bivalve mollusc growing areas) then sizing the buffer zone in consideration of the most likely worst-case condition is recommended.

If conditional management is considered, a buffer zone should achieve a minimum level of dilution at all times when the STW is operating as intended and the growing area is in the open status. As previously mentioned, prompt communication established in a conditional management plan is necessary during periods of degraded treatment such that the growing area can be placed in the closed status. It is also a good measure to validate closure thresholds and the management of a conditional area through assessing the potential impacts directly on bivalve molluscs in the growing area.

Sampling considerations

When developing a sampling strategy to assess STP efficiency with respect to virus reduction, the sampling strategy should be designed to assess viral performance under normal and challenged conditions and over a time period that is sufficient to capture seasonal, environmental and process related STW variability in performance. Thus, it is recommended that a minimum of fifteen (15) samples are collected no sooner than 2-week timeframe to properly assess the consistency and effectiveness of the treatment and at any stage in treatment that the STW may most likely fail. These samples should target conditions representative of the range of flows that are

expected for the works based on historical records. If the evaluation has identified factors that may affect the flow rate and/or the ability of the works to efficiently treat sewage than these factors should be considered in the sampling strategy. If the performance of the STW is shown to be significantly altered due to storm events than a more intensive storm event sample collection could be conducted. This could include: 1) A pre-storm sample to determine baseline conditions under dry weather and low flow rate at the STW which is typically a period when the treatment performance is the highest; 2.) During storm sample to capture the STW under wet weather and higher flow rate conditions which may be a period when the treatment performance is lowest; 3.) Post storm sampling to determining how long it takes for treatment efficiency to recover from the adverse conditions back to baseline conditions.

Further discussion of MSC sampling of bivalve molluscs is presented in Annex 11.

Additional factors to be considered in sizing a buffer zone

In addition to the type of classification adjacent to the STW and the determination for a STW failure in treatment additional factors should be considered in sizing of a prohibited buffer zone including: the volume flow rate of the STW; dispersion, dilution and build-up of contaminants in receiving waters; time of waste transport to the bivalve mollusc harvest area; and, decay rate of contaminants. These factors are discussed in more detail below.

(i) Volume flow rate of the STW

When conducting the dilution analysis, a range of effluent flow rates under all conditions (e.g. dry and wet weather) should be considered. If effluent flow rate data is available (such as through a permit) it is recommended that a 90 percent-tile daily flow rate is calculated from historical data and used in the dilution analysis in the determination of the dilution necessary to sufficiently dilute the loading under the 90 percent-tile flow conditions to the water quality criteria established for the growing area. When assuming a failure condition, it is recommended at a minimum that a failure loading over a 24-hour period is used unless site specific information such as gathered through the STW questionnaire (Annex 3) informs otherwise.

(ii) Dispersion, dilution, and build-up of contaminants in receiving waters

Simplified dilution analyses are provided below in Section 5 of this annex: these can be used to conservatively to establish a buffer zone conservatively sized based on a failure event if little or no information exists on dispersion, dilution and build-up of contaminants. Annexes 7, 8, and 9 provide information on factors to consider when conducting drogue studies, dye studies, or applying hydrodynamic models that may be used in the dilution analysis. These approaches may provide a more realistic determination of the buffer zone based on tidal factors that may influence the dispersion, dilution, and build-up of contaminants in the receiving waters. The establishment of a minimum sized dilution zone based on conditional management

should be based on one of the more accurate hydrographic determinations as presented in Annexes 7, 8 and 9 (and as discussed further in Section 5 of this annex) due to a shorter time of waste transport and lower level of dilution provided.

(iii) Time of waste transport to the bivalve mollusc harvest area

Any available information that may exist on the time of waste transport in the receiving waters should be considered (see Annexes 7, 8 and 9). Time of waste transport becomes more of a critical factor when conditional management is employed (see Section 4 of this annex). In determining the transport time of waste to a bivalve mollusc harvest area, peak current flows (such as might occur under spring or neap tides) at or near the outfall during ebb tide and flood tide should be considered for determining transport speed of effluent during possible failure conditions. Current velocity information may need to be generated if such information is not available or adequate for the area of the outfall. Current velocity information can be obtained from hydrographic dye studies, drogue studies, or current meter measurements taken from around the outfall. In many instances, particularly in geographic regions with large tidal amplitudes or swift tidal currents, the time of waste transport to the bivalve mollusc harvest area may be the more dominant factor in determining the size of the prohibited zone when considering both minimum dilution and time of waste transport.

(iv) Decay rate of contaminants

There are several conditions that affect bacterial and viral inactivation, including temperature, exposure to sunlight and sedimentation levels in the water (Burkhardt *et al.*, 2000; Lees, 2000; LaBelle, 1980). Scientists are unsure how long viruses remain viable in the marine environment, but it is likely to be weeks or months; enteroviruses have been found in marine sediments suggesting that sediments can be a source upon resuspension (Lewis, 1986). Moreover, bivalve molluscs have been found to retain viruses for much longer periods than they do bacteria (Sobsey, *et al.*, 1987; Dore and Lees, 1995; Dore, *et al.*, 2000; Shieh *et al.*, 2000). When a simple dilution analysis is conducted the most conservative assumptions should be applied which would be to assume zero decay in the environment given the potential longevity of viruses in the environment.

A10.3 BUFFER ZONES FOR MARINAS AND POPULATIONS NOT SERVED BY SEWAGE TREATMENT

Moored vessels such as marinas have the potential to contaminate adjacent shellfish growing areas. Thus, establishing a prohibited buffer zone that encompasses the area could potentially be impacted by vessel discharges is warranted. There are many factors that could be considered in the calculation of the necessary size of the buffer zone including: a slip occupancy rate for the marina; an actual or assumed rate of boats which will discharge untreated waste; an occupancy per boat rate (i.e., number

of persons per boat); seasonal changes in occupancy; and conservative assumptions of pollutant loading and depth of waters that could be impacted. An example of a marina buffer zone calculation is provided in Section 4 of this annex.

A similar buffer zone approach may also be taken for areas potentially impacted by populations that are not served by sewage treatment or where open defecation may be practiced. Understanding the total population that could be contributing to open defecation and the location that this may occur are the specific factors that would need to be understood.

A10.4 BUFFER ZONE DETERMINATION/DILUTION ANALYSIS

A10.4.1 *Simple dilution calculation for point source discharges*

If conducting a simple dilution analysis that does not account for tidal dispersion and pollutant build-up or when data is limited it is important to be as conservative as possible in the assumptions and assume worst case. The following is example of a simple dilution analysis assessing a point source and based on limited information and where a semi-circle radius buffer zone is employed (note: many shapes of the buffer zone may be utilized and GIS may be useful to shape to existing shoreline and coastal features).

$$A = \frac{V}{D} = \frac{\left(\frac{L}{G}\right)}{D} = \frac{\left(\frac{(F)\,(C)}{G}\right)}{D}$$

Where:

A = Area of Buffer Zone (Calculate)

V = Volume Impacted by Source (Calculate)

D = Depth of Water (Determine by Bathymetry Information)

L = Pollutant Load (Calculate)

G = Desired Water Quality Goal (Determine based on Classification Criteria to be met)

F = Flow Rate of Source (Determine from Permit Records or Flow Estimates)

C = Pollutant Concentration of Source (Assume worst case – literature values or measured)

PROS	CONS
> Little data needed > Simple calculations	> Tidal factors are not considered > May not produce realistic results
Key Factors: Need to account analysis limitations and use conservative assumptions to produce conservative results. Assume worst case pollutant load; If possible, assume 90%-tile pollutant source flows; If resource is suspended in water column assume the Depth of Water impacted is the depth that the product is suspended rather than depth of water to bottom; If product is natural set or on bottom assume depth at Mean Low Low Water when lowest level of dilution typically occurs; If pollutant discharged is a continuous or near continuous source consider using an additional Factor of Safety accounting for pollutant build-up as shown below.	

*Example Calculation: Sewage Treatment Works
with Flow Rate = 1 000 000 litres/day*

D = Depth of Water: 2 metres (based on Bathymetry Mean Low Low Water[10])

G = Desired Water Quality Goal: 14 M/100 ml (M=Microbial Concentration)[11]

F = Flow Rate of Source: 1 000 000 liters/day

C = Pollutant Concentration of Source: 1.4×10^6 M/100 ml (M=Microbial Concentration)[4]

Step 1: Determine Pollutant Load (L)

Pollutant Load (L) = Flow Rate of Source (F) × Pollutant Concentration of Source (C)

= (1 000 000 liters/day)(1000 ml/liter) × (1.4 M $\times 10^6$/100 ml)

= 1.4 M $\times 10^{13}$/day

Step 2: Determine Volume affected by Source (V)

Volume affected by Source (V) = Pollutant Load (L) / Desired Water Quality Goal (G)

= $(1.4 \times 10^{13}$ M/day) / (14 M/100 ml)(1 $\times 10^6$ ml/m^3)

= $(1.4 \times 10^{13}$ M/day) / $(1.4 \times 10^5$ M/m^3)

= 1.0×10^8 m^3

Step 3: Determine Area of Buffer Zone (A)

Area of Buffer Zone (A) = Volume affected by Source (V) / Depth of Water (D)

= 1.0×10^8 m^3 / 2 m

= 7×10^5 m^2

Step 4: Determine Size of Buffer Zone based on Area of Semi-Circle (example)

Area of Semi-Circle = $pr^2/2$

r^2 = $(7 \times 10^5$ m^2 x 2)/3.14159 = 4.46×10^5 m^2

$r = \sqrt[2]{r^2} = \sqrt[2]{446\ 000} = 668$ m

---

[10] The average of the lower low water height of each tidal day observed over a defined number of years (a period of 19 years is presently used in the United States and is termed the National Tidal Datum Epoch).

[11] The desired water quality goal (G) and the pollutant concentration (C) can be expressed as any suitable microbial pathogen or indicator (e.g. MSC). A generic value (M) is used here for illustration purposes.

FIGURE A10.4 **EXAMPLE OF A BUFFER ZONE BASED ON A SIMPLE DILUTION CALCULATION.**

Thus, the resulting buffer zone could be a semi-circle closure with a radius of 668 metres as shown in Figure 4.

NOTE: If the desired target is to determine concentration of microbial pathogen or indicator in bivalve molluscs, the above calculation could also be carried out to determine the concentration in bivalve molluscs assuming a conservative bio-accumulation factor. For example, if one were to use the 90 percenttile MSC value for influent (based on Pouillot et. al, 2015) of 214 000 PFU/100 ml as Pollutant Concentration (*C*) then the Desired Water Quality Goal (*G*) may be 0.5 PFU/100 ml if one conservatively assumes a 100-fold bio-accumulation (based on Burkhardt & Calci, 2000) which would then represent a concentration of 50 PFU/100 g in bivalve molluscs which may be the desired goal in bivalves.

A10.4.2 *Simple Dilution Calculation for Marinas or Open Defecation*

The following is example of a simple dilution analysis assessing a marina and based on limited information and where a semi-circle radius buffer zone is employed.

$$A = \frac{V}{D} = \frac{\left(\frac{L}{G}\right)}{D} = \frac{\left(\frac{(R)\,(P)}{G}\right)}{D}$$

Where:

A = Area of Buffer Zone (Calculate)

V = Volume Impacted by Source (Calculate)

D = Depth of Water (Determine by Bathymetry Information)

L = Pollutant Load (Calculate)

G = Desired Water Quality Goal (Determine based on Classification Criteria to be met)

R = Population Loading Rate (use NSSP recommended or other science based value)

P = Population/number of people (Determined)

PROS	CONS
> Little data needed > Simple calculations	> Tidal factors are not consider > May not produce realistic results
Key Factors: Need to account analysis limitations and use conservative assumptions to produce conservative results. Assume worst case pollutant load (concentration and number of people); If resource is suspended in water column assume the Depth of Water impacted is the depth that the product is suspended rather than depth of water to bottom; If product is natural set or on bottom assume depth at Mean Low Low Water when lowest level of dilution typically occurs.	

Example Calculation: Buffer Zone for Marina with 50 boats

R = Population Loading Rate: 2×10^9 M/Person/Day (M=Microbial Concentration)[4]

D = Depth of Water (e.g. MLLW): 3 metres

G = Desired Water Quality Goal: 14 M/100 ml (M=Microbial Concentration)[4]

P = Population = 50 boats × 2 people per boat (NSSP recommended value) = 100 people*

*Note: changes based on number of boats (assuming 2 people per boat)

Step 1: Determine Pollutant Load (*L*)

Pollutant Load (L) = Population Loading Rate (R) × Population (P)

$\qquad$ = $(2 \times 10^9$ M/Person/Day) × (100 People)

$\qquad$ = 2×10^{11} M/Day

Step 2: Determine Volume Impacted by Source (*V*)

Volume Impacted by Source (V) = Pollutant Load (L) / Desired Water Quality Goal (G)

$\qquad$ = $(2 \times 10^{11}$ M/Day) / (14 M/100 ml)(1000 ml/L)

$\qquad$ = 1.43×10^9 L per day

Step 3: Determine Area of Buffer Zone (*A*)

Area of Buffer Zone (A) = Volume Impacted by Source (V) / Depth of Water (D)

$$= (1.43 \times 10^9 \text{ L})(\text{m}^3/1000\text{L}) / (3 \text{ m})$$

$$= 4.76 \times 10^5 \text{ m}^2$$

Step 4: Determine Size of Buffer Zone based on Area of Semi-Circle (example)

Area of Semi-Circle $= \varpi r^2/2$

$r^2 = (4.76 \times 10^5 \text{ m}^2 \text{x } 2)/3.14159 = 3.03 \times 10^5 \text{ m}^2$

$r = \sqrt[2]{r^2} = \sqrt[2]{303\ 000} = 550 \text{ m}$

FIGURE A10.5   **EXAMPLE OF A BUFFER ZONE FOR A MARINA BASED ON A SIMPLE DILUTION CALCULATION**
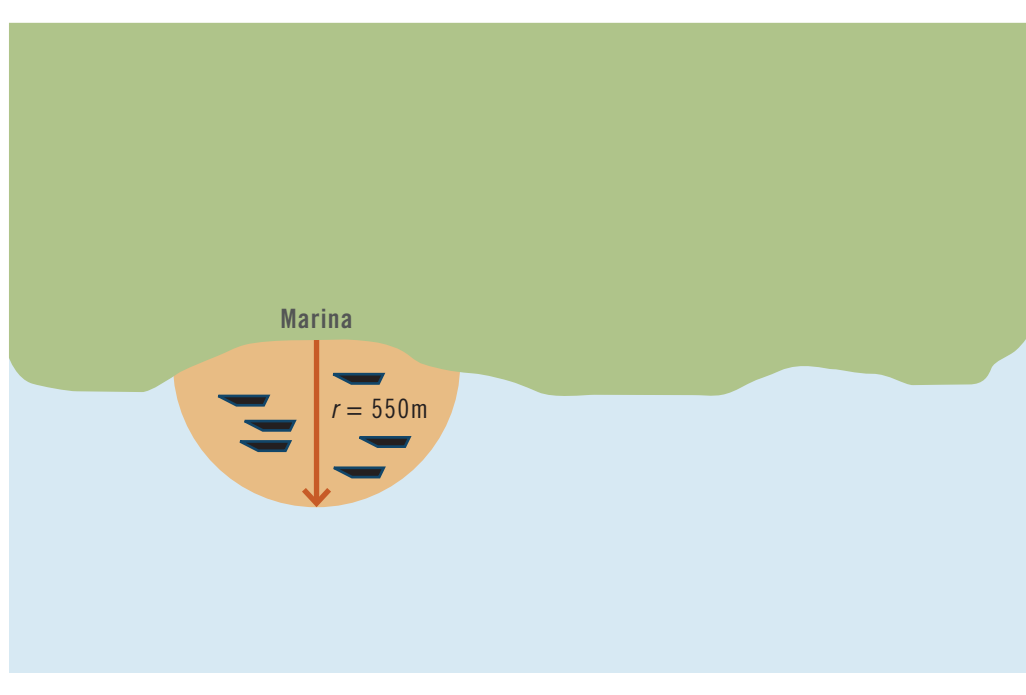
Thus, the resulting buffer zone could be a semi-circle closure with a radius of 550 metres as shown in Figure A10.5.

A10.4.3. *Simple dilution analysis with additional tidal information – point source discharges including overboard discharges.*

If tidal information is known such as through a drogue study or other tidal buoy station within proximity to the bivalve harvest area it may be possible to refine

the dilution analysis to reflect more accurately the area most likely impacted. The following is an example calculation of a simple dilution analysis within the necessary additional tidal information as gathered through a drogue study. The algorithm below is based on a pollutant dispersion model developed by Brooks and presented in Fischer et. al, 1979. This algorithm may also be applied to a STW to provide for a more realistic estimate of impact given consideration of the tidal conditions.

FIGURE A10.6   **DIMENSION NOTATION OF POLLUTANT PLUME**
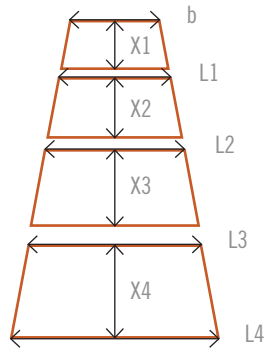
$$L = b(1+ 2/3(12\ \varepsilon\ /\ Vb)x/b)^{3/2}$$

Where:

L = width of the pollutant plume (calculated)

x = distance downstream (as determined via drogue study)

b = initial width of the pollutant plume (estimated based on width of source)

V = is the current velocity (as determined via drogue study)

ε = diffusion coefficient; and $\varepsilon = \varepsilon\ b^{4/3}$

α = dispersion coefficient: ranges from 9.1×10^{-4} m/s (conservative) to 9.1×10^{-5} m/s (less conservative)

PROS	CONS
> Tidal current, time of travel, direction considered > Produce more realistic results > Field work is not complex > Field work can be done with little resources and cost > Calculations are not complex if use spreadsheet	> May require field work > Calculations are more complex
Key Factors: Need to account analysis limitations and use conservative assumptions to produce conservative results. Assume worst case pollutant load; If possible, assume 90%-tile pollutant source flows; If resource is suspended in water column assume the Depth of Water impacted is the depth that the product is suspended rather than depth of water to bottom; If product is natural set or on bottom assume depth at Mean Low Low Water when lowest level of dilution typically occurs; If pollutant discharged is a continuous or near continuous source consider using an additional Factor of Safety accounting for pollutant build-up as shown below.	

Example Calculation: Buffer Zone for Fish Farm with 10 Workers

Fish (and bivalve mollusc) aquaculture operations may not have proper facilities for the collection and disposal of human waste from the workers. In addition, even if proper facilities are available, workers who have gastroenteritis may not be able to access the facilities in time and may dispose of faeces or vomitus to the marine environment. This example considers these issues.

Number of Workers on Fish Farm: 10

Depth of Water (e.g. MLLW): 3 metres

Volume Discharged (per person): 1 L/person

Concentration per person: 2×10^9 M/100 ml (M=Microbial Concentration)[4]

b = Initial Width of Pollutant: 3 metres

α = Dispersion Coefficient: 6.5×10^{-4} m/s

V = Current Velocity (determined via drogue study)

x = Distance travelled (determined via drogue study)

t = Time of travel (determined via drogue study)

TABLE A10.2 **EXAMPLE DROGUE STUDY RESULTS**

X - DISTANCE TRAVELED	Δ X	T - TIME	ΔT	V - CURRENT VELOCITY
METRES	METRES	MINUTES	MINUTES	METRES/SECOND
0	0	0	0	0.00
886	886	25	25	0.59
1 556	670	39	14	0.29
1 968	411	48	9	0.14
2 687	719	71	23	0.17
3 377	689	93	22	0.12
5 162	1786	145	52	0.21
6 173	1010	191	46	0.09

Table A10.2 shows the results of a drogue study conducted that measures the distance traveled and associated time of travel data (refer to Annex 11 for guidance on conducting drogue studies). The current velocity based on the drogue study can be determined in a spreadsheet. An example current velocity calculation for the distance traveled from 0 to 886 meters is shown below:

Step 1: Determine Current Velocity (V)

Velocity V = distance × (metre)/time of travel t (second)

= 886 m / (25 min)(60 sec/min)

= 0.59 m/s

Table A10.3 shows the algorithm results utilizing the drogue study data, computed current velocity, and the above assumptions (dispersion coefficient, initial plume width, and initial microbial concentration).

TABLE A10.3 **CALCULATED RESULTS BASED ON DROGUE STUDY AND POLLUTANT PLUME CALCULATION**

	X - DISTANCE	L - WIDTH	A - AREA	VOL - VOLUME	D - DILUTION	C - CONCENTRATION
	METRES	METRES	SQUARE METRES	CUBIC METRES		
	0					
1	886	31	1.51E+04	4.53E+04	4.53E+06	442
2	1 556	43	7.25E+04	2.18E+05	2.18E+07	92
3	1 968	61	1.75E+05	5.24E+05	5.24E+07	38
4	2 687	155	4.65E+05	1.40E+06	1.40E+08	14
5	3 377	213	1.09E+06	3.26E+06	3.26E+08	6
6	5 162	343	2.52E+06	7.57E+06	7.57E+08	3
7	6 173	851	6.21E+06	1.86E+07	1.86E+09	1

The calculations are best performed using a spreadsheet; and example calculations outlining the additional steps involved are provided below:

Step 2: Determine Volume Discharged by Fish Farm

Volume Discharged   = Volume Discharged per person × total number of ill persons

= (1 L/person) × (10 people) = 10 L

= (10 L)(1 m^3/1000 L) = 0.01 m^3

Step 3: Determine Diffusion Coefficient (ε)

Diffusion coefficient   = $\varepsilon = \alpha b^{4/3}$

=$(6.5 \times 10^{-4})(3)^{4/3}$ = 0.0028

Step 4: Determine Width of Plume (L)

Length 1 = b(1+ 2/3(12ε / Vb)x/b)$^{3/2}$

=3(1+2/3(12(0.0028)/(0.59 m/sec)(3 m)(886 m/3 m))$^{3/2}$

= 31 m

**Step 5: Determine Area of Plume
(Area 1 – as shown on Figure 6)**

Area 1 $= (b + L1/2) \times X1$ (refer to Figure 4)

$\qquad = (3 \text{ m} +31 \text{ m})/2 \times (886 \text{ m}) = 1.51 \text{ x}10^4 \text{ m}^2$

**Step 6: Determine Volume of Plume
(Volume 1 – as shown on Figure A10.6)**

Volume 1 $=$ Area 1 $\times$ Depth

$\qquad = (1.51 \text{ x}10^4 \text{ m}^2) \times (3 \text{ m}) = 4.53 \text{ x}10^4 \text{ m}^3$

**Step 7: Determine Dilution
(Within Volume 1 – as shown on Figure 10.6)**

Dilution 1 $=$ Growing Area Volume / Volume Pollutant

$\qquad = 4.53 \text{ x}10^4 \text{ m}^3 / 0.01 \text{ m}^3 = 4.53 \text{ x}10^6$

**Step 8: Determine Concentration in Growing Area
(within Volume 1 – as shown on Figure 10.6)**

Concentration $=$ Concentration of Pollutant / Dilution

$\qquad = 2 \times 10^9 \text{ M}/100 \text{ ml} / 4.53 \text{ x}10^6 = 442 \text{ M}/100 \text{ ml}$

**Additional Area Calculation – Area 2
(note slightly different from Area 1)**

Area 2 $\quad = (L1+L2)/2 \times X2 +$ Area 1 (refer to Figure A10.6)

$\qquad = (31+43)/2 \times (1556) + 1.51 \text{ x}104 \text{ m}^2$

$\qquad = 7.25 \text{ x}10^4 \text{ m}^2$

These calculations are carried out in a spreadsheet for remaining drogue study result data (as shown in Table A10.3). As shown in Table A10.3, the water quality goal of 14 M/100 ml would be met at 2 687 metres. The example outcome is illustrated in Figure A10.7.

FIGURE A10.7   **EXAMPLE OF A BUFFER ZONE TO TAKE ACCOUNT OF HUMAN POLLUTION ASSOCIATED WITH FISH FARMS**
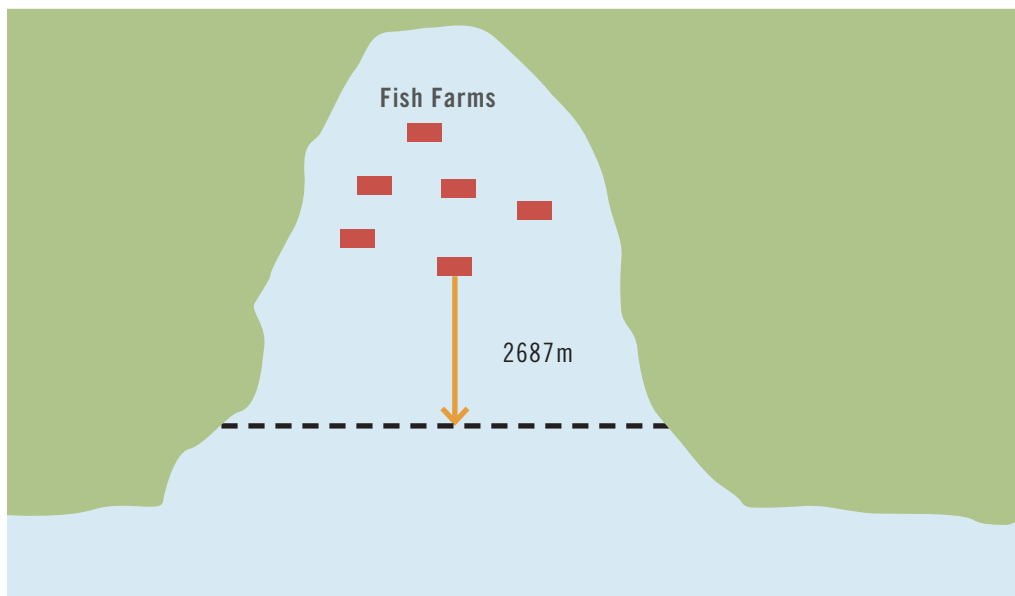
A10.4.4 *Additional Factor of Safety - Pollutant Build-Up for Poorly Flushed Estuaries*

In some poorly flushed estuaries pollutant build-up for continuously discharging sources such as a STW can be a significant factor (Goblick *et al.*, 2016, Campos *et al.*, 2017). To account for the potential for pollutant build-up as well as to provide for an additional factor of safety the following may be applied:

$$C_{ss} = C_{FT}(\frac{1}{1-R})$$

Where:

C_{ss} = Concentration at steady state

C_{FT} = Concentration on first tide

R = Fraction of pollutant returned each tide

Thus, if the above drogue-dispersion results were for a continuous point source such as small STW an additional factor of safety could be calculated as shown in the following example.

TABLE A10.4 **EXAMPLE CALCULATIONS – COMPARING CONCENTRATIONS AT STEADY STATE**

	X - DISTANCE	C - *CONCENTRATION*	C - *CONCENTRATION*
	FEET	FIRST TIDE	STEADY STATE
	0		
1	886	442	659
2	1 556	92	137
3	1 968	38	57
4	2 687	14	21
5	3 377	6	9
6	5 162	3	4
7	6 173	1	2

Example:

C_{FT} = 14 M/100 ml (as measured on first tide at the start of discharge)

R = 0.25 (33% of pollutant returns each tide)

Step 1: Determine Concentration (C_{ss}) at Steady State for 33 percent Rate of Return

$$Css = CFT\left(\frac{1}{1-R}\right) = (14\ FC/100\ ml)(1/1\text{-}0.33) = 21\ M/100\ ml\ \text{at steady state}$$

Utilizing the build-up factor and assuming that 33 percent of the pollutant returns each tide (not all of the pollutant is flushed out), the water quality goal (14 M/100 ml) at steady state isd met at >2 687 metres. A more precise estimate of where 14 M/100 ml is predicted at steady state could be determined by regression of the distance and pollutant concentration. Applying the build-up factor provides for an additional factor of safety and is recommended for poorly flushed areas.

A10.5 SUMMARY

The example problems presented in this Annex outline an approach that may be taken to assist with gathering the appropriate data and conducting an initial assessment of pollution source impacts to bivalve mollusc growing areas. However, it is highly recommended that any results from an initial assessment utilizing the methods presented here is validated through additional field surveys (e.g. microbiological conditions under worst case conditions – if possible) and/or hydrographic studies (e.g. drogue or dye tracer studies). Appendixes 7,8,9, and 11 provide additional insight in utilizing MSC, drogues, tracer dye, or hydrodynamic models which may be helpful to provide additional information and validation of buffer zone sizing. Additional information regarding the derivation of the recommended minimum dilution values based an assessment of STW efficiency and dilution in receiving waters is presented in Annex A10.1.

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RECOMMENDED DILUTION RATIOS FOR SEWAGE TREATMENT WORKS BUFFER ZONES

Recommended benchmarks to assist in the determination of STW performance and considerations for sizing the buffer zone are provided in Annex 10. These STW performance benchmarks (expressed as a log reduction of MSC) are for reference as it is recommended that a complete assessment of the discharge is conducted including determination of enteric virus loading to validate the dilution value used when sizing the buffer zone especially if consideration is given towards operating a conditional growing area based on the performance of the STW also described in Annex 10.

Minimum buffer zone dilution ratios for the given STW performance benchmarks presented in Annex 10 based on data from Pouillot *et. al*, 2015 are shown in Table A10.1.1.

These dilution ratios for sizing buffer zones are based on analysis of sixty-two (62) mechanical[12] sewage treatment works with a total of 595 samples of influent and effluent assessed in a meta-analysis of the reduction of norovirus and male-specific coliphage (MSC) concentrations. Utilizing the median and 90th percentile levels of MSC in influent and the overall log reduction of MSC achieved by mechanical STW considering no disinfection, chlorine, and UV were used to estimate the concentration of MSC in bivalve molluscs provided by the recommended dilution in growing area (provided by a buffer zone) and assuming 100-fold bioaccumulation (based on Burkhardt & Calci, 2000). Log reductions for a partially treated sewage are shown for illustration purposes with an assumed median log reduction of 1 and a 90th percentile log reduction of 0.5 assuming that only partial treatment of sewage was occurring. It should be noted that use of a 100 000:1 dilution is recommended for STWs in the US NSSP in the absence of data (NSSP, 2015).

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[12] Mechanical treatment referring to sewage treatment through means of mechanical components such as screens, grinders, settling tanks, blowers, etc., and achieving a combination of physical, biological, and chemical processes to treat wastewaters. Does not include aquatic treatment systems such as facultative lagoons and constructed wetlands.

TABLE A10.1.1 **RECOMMENDED MINIMUM BUFFER ZONE DILUTION BASED ON DATA FROM POUILLOT *ET AL.*, 2015**

MECHICAL WWTP	INFLUENT	MSC IN INFLUENT PFU/100ml[1]	LOG REDUCTION WWTP[1]	ESTIMATED MSC IN EFFLUENT PFU/100ml	MINIMUM DILUTION IN GROWING AREA	ESTIMATED MSC IN GROWING AREA PFU/100ml	ESTIMATED MSC IN BILVALVES PFU/100g[2]
Influent/raw	Median	158 000	0.0	158 000	350 000	0.45	45
Influent/raw	90% tile	214 000	0.0	214 000	350 000	0.61	61
Partially treated[3]	Median	158 000	1.0	16 000	100 000	0.16	16
Partially treated[3]	90% tile	214 000	0.5	68 000	100 000	0.68	68
No disinfection	Median	158 000	2.4	631	10 000	0.06	6
No disinfection	90% tile	214 000	2.1	1 811	10 000	0.18	18
Chlorine	Median	158 000	2.8	251	1 000	0.25	25
Chlorine	90% tile	214 000	2.5	620	1 000	0.62	62
UV	Median	158 000	4.3	8	300	0.03	3
UV	90% tile	214 000	3.9	27	300	0.09	9

[1] MSC in influent and log reduction for no disinfection, chlorine, and UV based on Pouillot *et al.*, 2015

[2] Estimated MSC in bivalve molluscs assuming 100-fold bioaccumulation based on Burkhardt & Calci, 2000

[3] Log reductions for partially treated sewage were assumed for illustration whereas others based on Pouillot *et al.*, 2015

Table A10.1.1 shows that for the recommended dilution ratios the estimated mean MSC levels in bivalves fall below 50 PFU/100 g which is below the US NSSP recommended level for MSC (NSSP, 2015). Additional data supporting the use a dilution ratio of 1 000:1 for conditional management adjacent to well monitored and high performing STWs is provided in US NSSP guidance (NSSP, 2015). Table 1 also shows that for the recommended dilution ratios the estimated 90th percentile MSC levels in bivalves fall below 125 PFU/100 g. Studies conducted in the UK have shown an association of norovirus and MSC in bivalve molluscs when MSC levels are 125 PFU/100 g or greater and were linked to official disease reports and were most frequently implicated in gastrointestinal illness. (Dore *et al.*, 2000).

It should also be noted that the validation of the sizing of the buffer zone can be conducted by the methods provided in Annexes 7 and 8 (hydrographic drogue or dye studies) and described in Goblick *et al.*, 2016. Additionally, buffer zone validation can also be achieved through microbiological testing of shellfish for MSC (and highlighted in Annex 11 describing uses of MSC) and/or enteric viruses and most comprehensively if conducted in conjunction with hydrographic studies as described in Goblick *et al.*, 2011 and Campos *et al.*, 2017.

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ANNEX 11

GUIDANCE ON THE USE OF MALE-SPECIFIC COLIPHAGE (MSC)

A11.1 INTRODUCTION

Bivalve molluscs have been associated with a wide range of microbiological illnesses, including those caused by bacteria, enteric viruses and parasites. Therefore, bivalve mollusc sanitation programmes require that bivalve molluscs and/or water, or both, be monitored for evidence of microbial contamination. Rather than analyse for a wide range of potential pathogens, countries normally monitor for microbial indicator organisms to assess the potential presence of pathogens in the marine environment. The indicator- based system is more proactive and cost effective than trying to monitor for multiple pathogens in the marine environment. The food safety goal is to measure harmless commensal bacteria, generally associated with the gastrointestinal tracts of warm blooded animals and shed in faeces in large quantities, as surrogates to reveal faecal contamination and the potential presence of pathogens in the media of concern. Commonly used indicator organisms are total coliforms, faecal coliforms and *Escherichia coli (E. coli)*.

However, the total and faecal coliforms and *E. coli* groups do not always satisfy the criteria for a good indicator. The faecal coliform group is not restricted to faecal habitats and may also be associated with vegetative detritus (Bagley and Seidler, 1977). Under certain circumstances faecal coliforms can multiply in the environment – thus causing false concern about pollution levels (Hood, 1983). Of greater public health concern is the fact that faecal coliforms and *E. coli* die off in the marine environment more quickly than the time required for virus inactivation. Enteric viruses that cause human illness, such as human norovirus (NOV) and Hepatitis A virus (HAV), often take weeks or months for inactivation to occur (Younger, Lee and Lees, 2002; CAC/CCFH, 2012). Therefore, it is possible that water samples will show low or non-detectable faecal contamination despite viable enteric viruses being present. In addition, enteric viruses can be bio-accumulated and eliminated or inactivated by molluscan bivalve molluscs unlike faecal coliforms and *E. coli*. When bio-accumulated by bivalve molluscs, viruses require a longer period for the animals to eliminate the viruses (CAC/CCFH, 2012).

This annex 11 applies to those programmes where the Growing Area Risk Profile has identified that one or more human enteric pathogens are relevant hazards to the growing area, and the subsequent sanitation programme is intended to address such hazards.

A11.2 WHAT ARE BACTERIOPHAGES?

Bacteriophages (commonly referred to simply as "phages") are viruses that infect bacteria. They can be found in all environments where bacteria grow, including in soil, water, and inside other larger organisms (animal, birds and humans) harbouring host bacteria. (Clokie *et al.*, 2011; Dutilh *et al.*, 2014; Díaz-Muñoz and Koskella, 2014). Phages are non-pathogenic and can only reproduce inside metabolizing bacterial hosts and are thus considered obligate intracellular parasites that cannot multiply independently in any environment outside of the host bacterial cell (Grabow, 2001; Brüssow, Canchaya and Hardt, 2004; Jończyk *et al.*, 2011). For replication to occur in a given environment, such as in bivalve molluscs and marine waters, their host must be viable in that environment (Grabow, 2001; Jofre, 2009) and susceptible to bacteriophage infection (Wiggins and Alexander, 1985; Woody and Cliver, 1995, 1997).

Male specific coliphage (MSC) is a very specific group of viruses that infect male-specific *E. coli* by attaching to the pili of the bacteria. MSC is similar to many enteric viruses in shape, transport and survival characteristics (EPA, 2015).

A11.3 WHAT ARE THE STRENGTHS AND WEAKNESSES OF MSC FOR BIVALVE MOLLUSC FOOD SAFETY?

MSC is found in faeces and sewage that contains *E. coli* and scientists have shown a relationship between MSC and human viruses in a number of marine and waste-water environments (Goblick *et al.*, 2015). International research has considered the use of MSC within bivalve mollusc sanitation programmes. Much of this work has been undertaken in Canada, Europe, Korea, North America and the United Kingdom. In 2015, the Joint USA-Canada Risk Assessment on Norovirus in Bivalve Molluscan Shellfish published a meta-analysis on the reduction of norovirus and MSC in waste-water treatment plants that had the potential to affect bivalve mollusc growing areas (Pouillot, 2015).

MSC is an easily detectable faecal coliphage, using a simple and relatively inexpensive test. This easy detection of MSC facilitates it use for faecal contamination detection in water and bivalve molluscs, which in turn can provide an insight into the possible levels of harmful human enteric viruses such as Norovirus. As a result, international researchers have concluded that due to MSC's similarity to enteric viruses, its abundance and ease of detection, it can be a useful indicator for monitoring what happens to enteric virus populations in water and bivalve molluscs. Therefore,

a number of international agencies have considered or are using MSC within environmental, public health and bivalve mollusc food safety programmes. (EPA, 2015; Younger and Lee, 2007).

However, as with any sentinel indicator, it also has weaknesses. Most studies relating to bivalve molluscs have been undertaken in relation to NoV and additional work is needed in relation to other pathogens that may be of concern, such as Hepatitis A and E. It is not a suitable indicator in situations where there is a low contribution of MSC levels into the marine environment. In such situations the MSC signal may be too low, or non-detectable, to predict the true food safety risk. Such situations may include, but are not limited to:

> low-flow sewage discharges from wastewater treatment plants from small communities;

> direct discharges from marine craft.

> discharges from small on-site-disposal, wastewater systems; or

> marine conditions affected by run-off from land contaminated with direct faecal deposition.

While there has been much international work done on the relationship between MSC and pathogenic viruses and bacteria, it has yet to be used comprehensively in all environments. Therefore, in addition to assessing the waste stream of the pollution source, consideration should be given to the latitude, temperature, salinity and any other relevant environmental features associated with the situation in which MSC is to be the target. If relevant data is not already available, it is recommended that baseline studies be undertaken to determine whether MSC will properly reflect inputs relating to human sources in the area under consideration (see Section 5 for more detail).

In summary, it is acknowledged that MSC has some limitations. However, MSC is a useful supplementary tool which can be used in conjunction with the traditional total coliforms, faecal coliforms and *E. coli* organisms. The benefit of MSC is that it is better indicator of enteric viruses present in human faecal contamination.

A11.4 SITUATIONS WHERE MSC HAS BEEN USED

Environmental and epidemiological research has showed that MSC is useful in many situations when used in combination with other information and tools. MSC has been used within the following bivalve mollusc sanitation tasks:

A11.4.1 *Characterizing Sewage Treatment Works (STW) performance*

There are now validated analytical methods for the analysis and enumeration of MSC from wastewater influent and effluent, as well as sewage-contaminated surface waters. With the use of these quantitative methods it is possible to directly determine

the quantity of MSC in wastewater, thus gaining information on the viral reduction efficiencies of wastewater treatment plants. The use of MSC in relation to sewage treatment works (STW) performance is most appropriate in situations where the treatment efficiency has inactivated other microbial indicators, for example in secondary and tertiary STWs. Please refer to Annex A3 for a checklist intended to assist in determining whether a treatment works is functioning correctly. More detailed information regarding STW efficiency testing can be found in Annexes A8 and A10.

A11.4.2 *Managing sewage pollution events*

The USA National Shellfish Sanitation Program (NSSP) has established an MSC standard for use following sewage spills or malfunctions at STWs. In such situations, it is considered that bivalve molluscs may be safely harvested for food consumption once the STW problems have been fully mitigated and there is evidence that the MSC level in bivalve molluscs has reduced to <50 pfu/100 grams or returned to the environmental background MSC levels.

A11.4.3 *Classification of bivalve mollusc harvest areas adjacent to STWs*

MSC is a tool which that can provide a better understanding about the source and strength of any sewage pollution impacting affecting the bivalve mollusc harvest area. There is now good evidence that MSC can be valuable in such situations because the potential viral impact of pollution sources would not have been evident by monitoring coliforms alone. MSC can also be used as a supplementary tool to refine the classification for bivalve mollusc areas. By measuring MSC levels in bivalve molluscs at the proposed harvest site it is possible gain a better understanding of dilution of any sewage effluent at the bivalve mollusc harvest site. For example, in some situations MSC has provided a better understanding of areas previously classified as 'Prohibited' (Category IV), proving that the bivalve molluscs are not impacted affected by one or more sewage discharges to a level deemed to be unacceptable, thus allowing the areas to be used for bivalve mollusc harvest.

A11.4.4 *Viral illness outbreaks and /investigations*

MSC has been used in response to growing areas implicated in illness outbreaks (McIntyre *et al.*, 2012). When used in addition to bacterial indicators and the specific outbreak pathogen (e.g. NoV) the extra information can assist with the outbreak investigation. MSC detections in the environment or shellfish may indicate a relatively fresh sewage source, increasing the possibility that a high ratio of NoV detected may be viable. However, the presence of NoV combined with MSC absence may indicate that the source is not linked to a large sewage source. Instead, the source could be a small overboard discharge (vomit or faeces from an ill individual) or alternatively, the source of sewage is no longer fresh (and thus less likely to be viable).

A10.4.5 *Relay operations*

Relaying is an internationally recognized and permitted practice whereby bivalve molluscs are transferred from mildly polluted areas to a natural clean seawater site. Long-term relaying over a period (a minimum of three weeks to several months), is used to remove viruses. For such long-term relay operations, when appropriate based on the factors discussed in Section 3, MSC may be used to verify that the bivalve mollusc have adequately cleansed at the completion of the stipulated relay period. For example, the recommended target of <50 pfu/100 grams (or acceptable established background level) can be used under the US NSSP to verify that the natural cleansing process has been effective. The period required for removal of specific viral pathogens may vary with the pathogen, bivalve species and environmental conditions.

A11.5 INFORMATION ON WHEN AND HOW TO SAMPLE FOR MSC

A11.5.1 *STW Characterization through sampling fluids*

See Annex 6 for further information on how to fully characterize the efficiency of a STW. MSC is best used to assess the performance of STW incorporating secondary and tertiary treatment where the concentration of the traditional bacterial indicators is reduced more than the concentration of viruses. In all other sewage situations, including raw and primary treatment, the bacterial indicators may still be appropriate.

For STWs incorporating secondary and tertiary treatment, the following MSC sampling principles should be used.

Sampling Plan

It is recommended that a minimum of fifteen (15) samples in total are be collected during at least five (5) sampling events (i.e. a minimum of three (3) samples per event) are undertaken in order to properly assess the consistency and effectiveness of treatment. These sampling occasions should target all diurnal, seasonal and environmental conditions and be representative of the range of flows that are expected for the works, based on historical records. If the evaluation has identified environmental factors that may affect the flow rate and/or the ability of the works to efficiently treat sewage, or both, then these factors should be considered in the sampling strategy. For example, if the evaluation reveals that the design capacity of the STW is exceeded frequently then it is recommended that at least one-third of the samples collected should be under these conditions. In general, precipitation events or snowmelt, seasonally high groundwater levels into a collection system with inflow or infiltration issues, or any other factors that might influence the treatment performance of the works should be considered. An effective strategy could be to collect approximately one-third of the samples before a storm event, a third of the samples during a storm event, and a third of the samples following

the storm, resulting in an even distribution of samples and capturing a full range of environmental and STW flow conditions.

Sample collection

Samples should be taken of the influent to the works (prior to any stage that may result in a reduction in microbial content) and of the effluent after the final stage of treatment (and preferably immediately prior to the discharge point). Sample access points may be provided for use by the works staff and/or environmental regulator. In terms of sample collection, grab samples are most feasible. However, due to the large variability in pathogen levels and residence time through the works a composite sample is preferable. An automated sampler can be used to collect hourly samples that are composited into a single bottle. If a diurnal cycle at the works is observed (periods of high flow and low flow based on usage throughout the day), sampling times for compositing samples can attempt to capture separately these time periods. Diurnal flow changes in the works can vary widely depending on size of plant, population served, catchment size, and the use of the works (sanitary, industrial, storm water, etc.). Sample size should reflect the types of analyses to be conducted.

Sample analyses

Recommendations for on methods for microbiological analyses are given in Section 4.3.8 of the main document.

Assessing viral reduction efficiency of STW

The efficiency of the works can be assessed considering the log reduction in the level of viruses through treatment determined from the influent and effluent samples as follows:

Viral performance index = Log (influent sample) -Log (effluent sample)

Geometric mean and tenth percentile values from the complete dataset used for analysis can be compared with the recommended benchmarks above to determine into which class the STW falls.

A11.5.2 *MSC Sampling of Shellfish*

Sampling of Shellfish at Harvest Site

MSC analysis is one tool that can be used to assist in determining whether bivalve molluscs are excessively contaminated with STW effluent. Such testing might be undertaken after a STW emergency event, and/or a sewage spill, to determine when the harvest area can re-open. Alternately, MSC might be used to verify that all sampling sites within the harvest area are not adversely affected by sewage pollution under normal environmental conditions. In either case, the recommended MSC

threshold of 50 pfu/100 grams can be used, unless the responsible authority has established an alternative baseline background MSC level. Such a background level could be determined by undertaking fortnightly sampling under the range of seasonal and environmental conditions expected in the area. This may be achieved by taking additional animal samples for MSC analysis at the same time as primary monitoring samples for faecal indicator bacteria are taken.

However, it is important to note that MSC levels of 100 pfu/100 g or greater in bivalve molluscs have been epidemiologically linked to outbreaks of NoV. Thus, if levels of MSC in bivalve molluscs are found to be 100 pfu/100 g or greater during baseline sampling in a Category I growing area, efforts should be made through the sanitary survey to determine if levels above 100 pfu/100 g derive from background or are due to a sewage source. Such elevated MSC levels may indicate that the area is subject to at least intermittent contamination potentially containing human enteric virus at levels that may be unacceptable, and thus appropriate post-harvest treatment may be necessary to reduce the risk from such contamination.

MSC should be used in conjunction with bacterial indicators to verify the status of bivalve molluscs. It is important that all the indicators used comply with any regulatory requirements before re-opening a growing area after a sewage pollution event. Samples should be collected from all the routine sampling sites within the harvest area to verify that the entire area has recovered from the contamination event.

Sampling within the greater catchment

When undertaking the catchment sanitary assessment, MSC analysis can help locate sewage contamination sources, such as leaky sewage collection system infrastructure, lift station failures, illegal discharges, or cross-connections to between storm drains and community septic system overflows. Utilizing sampling of naturally established bivalve molluscs located along the shoreline can be helpful in determining areas where a sewage signal may be present. If there are no established shoreline bivalve molluscs it may be possible to deploy bivalve molluscs in cages in targeted areas.

Sampling shellfish linked to viral illness outbreaks and investigations

During a human enteric viral illness outbreak (e.g. NoV, HEP-A), bivalve mollusc samples within the growing area associated with the outbreak can be collected and analysed for the target pathogen and for MSC, in addition to bacterial indicators used to classify the growing area. Sample results can be used to determine if the source of contamination potentially originated from within the growing area, as well as to assess the sanitary quality of the growing area at the time of sampling. Sampling should be undertaken at the routine faecal indicator monitoring points, plus additional points located within the bivalve resource at those locations identified as being likely to be affected by sewage from any sewage sources identified as possibly contributing to a contamination incident (e.g. a sewage works that has experienced a recent bypass event;, occurrence of emergency or storm-related discharges;, or breakage in a sewage collection (sewerage) system. Ideally, samples should be taken at each location on each of three separate days. Analysis may be undertaken for the

normal faecal indicator used in the programme, as well as for MSC (see below for comments on monitoring for specific pathogens).

Samples should consist of at least ten separate animals of each relevant harvested species for each microbiological analysis to be performed (a greater number of animals from smaller species may be needed to obtain sufficient material for analysis – see Annex 10). Recommended microbiological methods are given in Section 4.3.8 of the main document.

MSC detected in bivalve molluscs (combined with routine water quality monitoring results) can be used to locate potential "hot spot" areas within the growing area, which might lead to a portion of the watershed catchment that may have contributed to or contained the source(s) responsible for the elevated microbiological results and, potentially, the outbreak. Similar to the strategy used in utilizing MSC to characterize the bivalve mollusc catchments discussed above, samples collected in the growing area can help target sanitary survey activities in the catchment that should be conducted following an outbreak to try to establish a link between the outbreaks and potential sources that may have contributed to the event.

It is important to note that testing of bivalve molluscs for MSC from a potentially incriminated harvesting area supplements, but does not replace, testing for the specific pathogen for investigative purposes. As previously mentioned, there can be situations where MSC may be suitable to locate pollution sources in the catchment but not suitable for assessing the human enteric viral risk of bivalve molluscs in the growing area. For example, although MSC may be found in effluent from a septic tank, the levels can vary greatly, depending on the contributing population.

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ANNEX 12

EXAMPLE SAMPLING PROTOCOL

A12.1 INTRODUCTION

Samples may be taken within a bivalve sanitation programme as:

> part of planned primary or ongoing monitoring, to help define conditional classification criteria;

> as part of a shoreline survey; or

> to support investigations (e.g. related to the activation of an expected or unexpected event management plan) – see Table A12.1.

Samples taken for all of these purposes form part of the official programme and need to be taken according to standard protocols. There are some differences in approach for sampling for these different purposes and these differences are identified within the protocol.

TABLE A12.1 **SAMPLES THAT MAY BE TAKEN AS PART OF A BIVALVE MOLLUSC SANITATION PROGRAMME**

SANITATION PROGRAMME COMPONENT	TYPE OF SAMPLE			
	SEAWATER*	BIVALVE MOLLUSCS*	FRESHWATER	EFFLUENT
Shoreline Survey	√	√	√	√
Primary Monitoring	√	√		
Ongoing Monitoring	√	√		
Investigations	√	√	√	√

* Whether water or bivalve samples are taken during primary or ongoing monitoring will be determined by whether classification is to be based on results from faecal indicator analysis of water, bivalves, or both. Whichever approach is taken, samples of both water and bivalves will usually be taken during the shoreline survey and during any investigations.

A12.2 TRAINING

Staff undertaking sampling for any purpose within the bivalve mollusc sanitation programme should be trained in relevant sampling techniques. For shore-based sampling, this should include general safety training for shore-based fieldwork. For boat-based sampling this should include general safety training on working on boats in appropriate marine and/or estuarine locations: this may need to respect specific local regulatory requirements. Those driving boats should be qualified to do so, and should be competent in navigation techniques and maritime communication procedures.

Where another agency or body undertakes the sampling by formal agreement (including under contract) the responsible authority should verify that the staff of that agency or body have been properly trained. Periodic audits should be undertaken to ensure that the required procedures are being followed.

A12.3 HEALTH AND SAFETY

Fieldwork can be unpredictable and dangerous. Weather and sea conditions can change rapidly. It is the responsibility of both management and staff to reduce the risks as far as is practically possible. A risk assessment should be prepared prior to any sampling being undertaken. Individual locations may represent additional problems or risks which will need special additional consideration to ensure safety. Staff working on the shore or in boats should continually assess the risks as they go, taking account of changes in conditions (e.g. weather) that would affect the overall risk of the work. If the risks increase, then the field team should consider whether the work plan should be changed to reduce the risk. If necessary, the work may need to be stopped and continued at a later date.

Two or more people should normally undertake sampling as a safety precaution. Sampling at specified locations may be undertaken by a lone worker if this is allowed under the local health and safety system and if the additional considerations are addressed in the risk assessment. Contact systems should be used to notify the office (or other contact point) of progress.

Boats that are used for sampling should be appropriate to the location (lagoon, estuary, inshore, offshore), the expected weather conditions and sea state, the number of people on board and the sampling activities to be performed. Sampling may require the use of special equipment. e.g. dredges for benthic stocks or to winches to raise longlines. Appropriate safety equipment should be carried on the boat: this may be specific in local regulations. Life jackets should be provided and worn at all times.

Boots and gloves appropriate to the task are required. In general, sturdy boots with soles that will resist penetration by sharp objects are required for shore-based work. Boots with non-slip soles are best for rocky surfaces and working on boats. Waterproof (Wellington) boots may be required if it is necessary to wade into the

water to collect samples. The use of waders should be avoided where possible as they encourage staff to enter water that is too great a depth. There is also a risk of them filling with water and causing the wearer to sink. If they are supplied, relevant training should be given and their use should be specifically addressed in the risk assessment. While sterile disposable gloves are usually used for sampling, sturdy protective gloves may be necessary for handling some equipment on boats and for handling bivalve species that have sharp edges. Hard hats should be worn on boats with overhead equipment. Other clothing should be appropriate to the expected conditions and intended activities.

Contact with polluted water and sewage discharges can result in infection. Unpolluted water can also be the source of some bacterial infections and parasite infestations. Infections may also result from the ingestion of such waters and material from discharges, either directly or via food contaminated by hands. Discharges may contain chemicals or radionuclides which may also cause harm via the skin or by ingestion. Disposable gloves should be worn when taking samples and hands should subsequently be cleaned before touching drinks containers or food.

Also see the "Access and physical safety" and "Personal care" subsections of Annex 4 (Shoreline Survey Checklist).

A12.4 PLANNING OF SAMPLING OPERATIONS

Sampling should be carefully planned so that staff have the correct information on planned sampling locations, required sample types and analyses, any special considerations such as targeting particular times or tidal states and so that they take the correct equipment for the sampling operation. Annex A12.1 shows an example summary information sheet to be used in planning sampling events.

A12.5 COMMUNICATION WITH LABORATOR(Y/IES)

Depending on local capabilities and arrangements, different laboratories may be used for different specimen types or different types of analysis. In such cases, relevant communication should take place with each laboratory.

The laboratory should be kept informed of planned sampling activity. It should be ensured that the planned activities will mean that the samples will arrive at the laboratory on a day and at a time that they can be accepted and processed.

The laboratory should be informed of the number and type of samples that are planned (e.g. bivalves, seawater, freshwater, sewage) and the type of analysis required.

Where the full extent of sampling is not known at the outset, e.g. for a shoreline survey or investigation, the general plans should be communicated to the laboratory in advance and then an update given of the actual numbers of samples of each type taken around the time of sample dispatch.

See Annex 11 (Example Sample Transport Protocol) for further considerations.

A12.6 SAMPLING LOCATIONS

Samples for primary or ongoing monitoring should be taken from within the specified tolerance of the planned sampling locations. If this is physically not possible, e.g. due to lack of bivalve resource at an intended bivalve sampling location, or due to the tide being out at an intended water sampling location, a sample should be taken as close as possible to the intended sampling point. The actual sampling location of these should be recorded together with a note that this differed from the intended location. The person responsible for managing the monitoring programme should also be informed of the problem in case there is a need to revise the sampling plan(s) for the growing area.

Samples taken during shoreline surveys and investigations may be taken from planned locations, from impromptu locations identified in the field in response to an observation (e.g. a watercourse, a discharge or evidence of pollution), or from a combination of planned and impromptu locations. With respect to planned locations, where samples cannot physically be taken from the intended locations, they should be taken from the nearest possible location to the intended sampling point and the actual sampling location recorded.

The following information should be taken into the field for all planned samples:

> sample point identifier (for planned samples taken for primary or ongoing monitoring; this may be a name or a code);

> type of sample (including species if bivalves);

> size of sample;

> date and, if appropriate, time or prevailing conditions for sampling;

> intended sampling location;

> tolerance around intended sampling point (radius from the intended point within which a sample must be taken if it cannot be taken at the exact point); and

> any other special instructions.

This information may be taken in hard copy (preferably laminated) or within a waterproof computer or other electronic device. This may also be accomplished by loading the locations as waypoints in a Global Positioning System (GPS) handset and associating the other relevant information with these waypoints.

Planned sampling locations should NOT be entered into the actual sampled location fields of sample request forms or other sampling record systems as this may result in a failure to record the actual location of sampling. The actual location from which the sample is taken must be recorded.

A12.7 EQUIPMENT – GENERAL

> GPS handset;

> Heavy-duty polythene bags;

> Cable ties;

> Sample labels (waterproof);

> Disposable gloves;

> Disinfectant wipes;

> Permanent marker (waterproof);

> Validated cool-box;

> Freezer packs (number for each box as per the validation);

> Newspaper or foam spacers for cool box;

> Sample submission forms (see Section 5).

See also additional equipment requirements given in the bivalve and water sampling sections.

A12.8 SAMPLE TRANSPORT

Samples should be transported according to the protocol given in Annex 11. If there is to be a delay before transferring bacteriological or other temperature critical samples to a cool box for transport, then arrangements need to be made for keeping the samples cool between sampling and transfer to the transport cool box.

A12.9 BIVALVE MOLLUSCS

A12.9.1 *Additional equipment*

The following additional items are required for bivalve sampling:

> Heavy duty gloves - for handling species such as *Crassostrea gigas* (Pacific oysters) that have sharp edges to parts of the shells;

> Scrubbing brush - for removing material such as mud from the outside of the shells;

> Bucket (collapsible variety is easiest for transport) – for containing water when water is not available at the immediate sampling location;

> Supply of potable or clean sea water – for cleaning the outside of the shells when water is not available at the immediate sampling location.

A12.9.2 *Bivalve mollusc species*

The species to be sampled for primary and ongoing monitoring should be specified in the sampling plan. This may be sampling all of the species harvested (or intended to be harvested) at a specific location or the sampling of one or more species. This is decided by the programme managers and the requirements given in the sampling plan should be followed.

For sampling undertaken during shoreline surveys and investigations, the species may be specified for planned samples. However, for the planned sampling of wild stocks and impromptu sampling during these activities it may be necessary to take samples of whatever species is available at the location. It may be useful to sample more than one species at a location as it may not be possible to predict which will give higher results on one occasion.[13]

A12.10 BIVALVE NUMBERS

The following minimum numbers of animals per sample should be submitted to the laboratory for analysis for faecal indicator bacteria (taken from ISO 6887-3) (Table A12.2).

TABLE A12.2 **RECOMMENDED NUMBERS OF ANIMALS PER SAMPLE FOR FAECAL INDICATOR BACTERIA ANALYSIS**

SCIENTIFIC NAME	COMMON NAME (ENGLISH)	NUMBER
Pecten maximus	Atlantic great scallop King scallop	12 – 18
Aequipecten opercularis *Chlamys (Aequipecten) opercularis (Linnaeus)*	Queen scallop	18 – 35
Crassostrea gigas	Pacific oyster	12 – 18
Ostrea edulis	European flat oyster Flat oyster	12 – 18
Mercenaria mercenaria	northern quahog = Hard clams	12 – 18
Tapes philippinarum	Manila clam	18 – 35
Ruditapes decussatus	Grooved carpet shells	18 – 35
Spisula solida	Thick trough shells	35 – 55
Mya arenaria	Sand gapers	12 – 18
Ensis spp.	Razor clams	12 – 18
Mytilus spp.	Mussels	18 – 35
Cerastoderma edule	Cockles	35 – 55
Donax spp.	Bean clams	40 – 70

[13] Species vary in their kinetics of uptake and depuration and also in the maximum levels reached in a particular situation. These characteristics may also be affected by environmental factors such as temperature and salinity.

For other species, or other analyses, the minimum number should be determined according to the following steps:

> Determine, in liaison with the testing laboratory, the minimum number of animals of mature size that will yield sufficient material for the analys(is/ses) in question (a minimum of ten animals is specified in ISO 6887-3 for microbiological examinations – this is intended to reduce variability due to differences in concentration of the target microorganism among individual animals).

> Add an extra 10 percent of animals to yield the sampling requirement in order to allow for a proportion of animals becoming moribund during transport and storage.

> Increase this proportion if a sampled species shows greater than 10 percent morbidity during transport.

A12.11 SAMPLING PROCEDURE

Prior to the sampling date: freeze sufficient cold packs to chill the numbers of samples to be collected. If multiple sites are being sampled, it may be necessary to take a separate cool box for each site.

For some analyses, such as some virological, molecular or chemical methods, it may be permissible to submit the bivalves to the laboratory in a frozen state. This should only be done in arrangement with the receiving laboratory and where the effect of freezing has been shown not to affect the outcome of the analysis/analyses. Samples for conventional bacteriological analyses MUST NOT be frozen.

Where possible, bivalve samples should be collected using the same method typically used for commercial harvesting in an area.

Prior to collecting samples, ensure all sampling materials are clean and to hand. Wipe hands thoroughly with an alcohol-based hand sanitizer and don disposable gloves.

Whenever possible, collect animals that are of harvestable size. Juvenile or undersize individuals may concentrate bacteria at a different rate than full size individuals and this could affect the result.

After bivalves have been removed from the water and have closed, any mud or sediment adhering to the shells should be removed by scrubbing/rinsing with seawater from the immediate vicinity of the sampling site. Do not immerse the bivalves during the cleaning process. Allow the bivalves to drain. Do not re-immerse bivalves after cleaning.

Place the collected sample in a heavy-duty, food grade, unused polythene bag and seal with a cable tie. Place this bag in a second bag and seal this bag, preferably with a tamper proof seal (otherwise a cable tie may be used). Label clearly with the species, sampling location, date and time taken or unique sample identifier number that is replicated on the sample submission form. Complete a sample submission form (see Section 2.8). The coordinates of the ACTUAL sampling location MUST

be recorded to at least 10 m accuracy using GPS – DO NOT use the identifier of the planned sampling location instead.

A12.12 WATER

A12.12.1 *Sample size*

Seawater samples for the enumeration of faecal indicator bacteria should be a minimum of 150 ml. Freshwater samples and samples of discharges for faecal indicator bacteria (e.g. taken during a shoreline survey) may be collected in sterile 30 ml universal bottles. For other analyses, ascertain with the laboratory the minimum size of sample needed for the analys(is/ses) and ensure that the sampled volume is at least 50 percent greater than this.

A12.12.2 *Sample containers*

For microbiological analysis, samples should be collected in sterile glass or plastic bottles or bags suitable for the transport of liquid samples. The material of which they are made should not adsorb or inactivate the target micro-organisms. For other analyses, advice should be sought from the receiving laboratory as to the material from which the bottles or bags should be made. The laboratory may require the addition of a preservative prior to transport of samples for some chemical determinands. The receiving laboratory may be able to supply appropriate bottles, bags or preservative.

A12.12.3 *Other equipment*

> Sampling pole
> Water sample pots or bags of an appropriate size (e.g. 180 ml for seawater samples and 30 ml for freshwater and discharge samples for faecal indicator bacteria).

A12.12.4 *Sampling procedure*

When collecting samples, care should be taken to follow good hygienic practice to avoid contaminating both the sampler and the sample. Disposable gloves should be worn when collecting samples to avoid contaminating the sample container (these gloves should be stored in such a way that they do not become contaminated themselves during the sampling trip). Care must be taken not to touch the internal surfaces or rim of the sample container or the inside of the lid: also protect the inside of lid from contamination from other sources.

Samples should be taken up-current of the sampler to prevent contamination from disturbed sediment, the sampler's body or the sample bottle itself. Water samples should be taken from the middle of the water column, avoiding surface and near sediment samples as much as possible. For samples taken from a boat, it is preferable for the vessel to be pointed into the direction of any flow and the sample taken from the bow (upstream of the boat).

Sampling by pole

For samples taken from a boat, an extendable sampling pole should be used. Prior to use, the pole should be wiped with a disposable disinfectant wipe and then rinsed in the sea near the sampling point. A pole may also be used for sampling from a shoreline to allow the sampler to access deeper water away from any contamination arising from the sampler's boots and clothing or from watercourses where it is not possible or safe for the sampler to wade in. Samples should always be taken upstream from the sampler.

Fix the sterile container to the clamp at the end of the pole. Remove the cap just before collecting the sample, taking care not to contaminate any inside surfaces. The cap may be placed in a clean plastic bag or held while sampling, so long as the inside surface of the cap is not contaminated. Extend the handle until the sample container is over water at least 1 metre deep and up-current of the boat and sampler. Invert the container so that it enters the water opening first, and lower it to a depth of about 30 cm. Turn the container 180° and wait 30 seconds for the container to fill. Leave a small air space at the top of the container to allow for shaking to mix the sample. Remove from the water and replace the cap.

For unknown discharge flows, the depth may be significantly less than 1 m. Where possible, a water sample should be collected from the midpoint of the deepest part of the flow, taking care to avoid stirring up any solid material on the bottom.

Sampling by hand

Where the sampling point is accessible safely by foot, samples may be taken by hand. If possible, take the sample from a point where the water is approximately 1 m deep. This will allow for easier sampling of the water column. Wade out to the sampling point, uncap the bottle taking care to not contaminate the cap or inside surfaces, face into the current, lower the container inverted to a depth of 30 cm and then turn the container upright to allow it to fill. Remove from the water and leave a small air space above the sample to allow for mixing later. Re-cap the bottle as soon as possible.

Each bottle should be labelled, either with a unique sample identification number, or the sample point identifier and the date and time. Bottles should be carefully sealed and placed in a cool box. See Annex 13 (Example Sample Transport Protocol) for further details on cool box packing.

A12.13 SAMPLING RECORDS

Samplers should keep records of sampling activities in addition to completing sample submission forms. These records may be completed electronically or in hard copy. Example record sheets for the sampling of bivalves and seawater are given in Annexes A12.2 and A12.3 respectively.

A12.13.1 *Sample submission forms*

Sample submission forms should contain all relevant information agreed with the monitoring programme manager and the receiving laboratory. as a minimum this should include:

> sample point identifier (for planned samples taken for primary or ongoing monitoring);

> type of sample (including species if bivalves);

> date of sampling;

> time of sampling;

> location of sampling (latitude/longitude [as WGS84] or relevant national grid co-ordinates; accurate to at least 10 m);

> required analyses; and

> any specific observations of interest (e.g. operating discharge, large numbers of animals or evidence of pollution; abnormal weather conditions; etc.).

An example sample submission form is given in Annex A12.13.4.

A12.13.2 *Storage of samples prior to transport*

All water samples should be securely fastened and stored upright to prevent leaks. After sampling, all samples to be transported to the laboratory in coolboxes should be placed under cooled conditions as soon as possible. If there may be a delay in transferring to a coolbox, for example during extended sampling operations such as a shoreline survey, samples may be placed in an insulated backpack containing cool packs. The samples should not come into direct contact with the cool packs.

A12.14 SUPPORTING TEMPLATE FOR SAMPLING RECORDS

A12.14.1 *Example planning sheet for sampling operations*

PROGRAMME/ PROJECT/ PURPOSE	NATURE	SAMPLE TYPE	SAMPLING POINT IDENTIFIER	SITE	SAMPLING DATE	SAMPLE TIME	SAMPLERS	ANALYSES	METHOD OF TRANSPORT	LABORATORY
Ongoing monitoring	Shore	Bivalve	MORBIV09	Sidi Boughaba	2015-08-17	Low tide	Omar Hassikou, Rachida Charof	Microbiology – *E. coli* Biotoxins	Service vehicle	INRH, Agadir
	Shore	Bivalve	MORBIV23	MyBous-selham	2015-08-24	Low tide	Omar Hassikou, Rachida Charof	Microbiology – *E. coli* Biotoxins	Service vehicle	INRH, Agadir

A12.14.2 *Example sampler record sheet: bivalves*

Date:……………………………………… Time:……………………………………………..

Programme:……..…………………………………………………………………………..

Sampler(s)……..…………………………………………………………………………....

Site identifier……..…………………………………………………………………………..

Site name……..…………………………………………………………………………....

Sampling equipment……..…………………………………………………………………

Bivalve species……..………………………………………………………………………….

Depth……..…………………………………………………………………………...……

Tidal state……..……………………………………………………………………………

Observations: ……..………………………………………………………………………..

…………………………………………………………………………………………………

Sample identification number……..…………………………………………………………

A12.14.3 *Example sampler record sheet: seawater*

Date:……………………………………… Time:……………………………………………..

Programme:……..…………………………………………………………………………..

Sampler(s)……..…………………………………………………………………………....

Site identifier……..…………………………………………………………………………..

Site name……..…………………………………………………………………………....

Sampling equipment……..…………………………………………………………………

Depth……..………………………………………………………………….. ……

Tidal state……..……………………………………………………………………………..

T°………………..pH………………..O_2………………Salinity (ppt)…………………………

Observations: ……..………………………………………………………………………..

…………………………………………………………………………………………………

Sample identification number……..…………………………………………………………

A12.14.4 *Example sample submission form*

Agency name:
Country of State:

Growing Area: _____ Location: _____ Cuntry of municipality: _____

Date: _____ Start time: _____ End time: _____ Name of sampler: _____ Name of harvester: _____

Sample identification number	Sample point identifier	Actual sampling location	Time (24-hour format)	Type od sample				Physicochemical parameters					Type of sample	
				Seawater	Freshwater	Effluent	Bivalve molluscs (species)	Temp (°C)	pH	Oxygen	Salinity		Water/ effluent	Bivalves
												Total of Fecal coliforms		
												E. Coli		
												Salmonella spp.		
												V. parahaemolyticus		
												V. cholerae 01 or non-01		
												V. vulnificus		
												Heavy metals: Cd, Pb, Hg, As		
												Pesticides		
												Saxitoxin		
												Domoic acid		
												Okadaic acid		
												Others		

Type of preservation: Icepack ▭   Electric cooler ▭

Remarks:
- Rainfall
- Tide
- Pollution

- Surf
- Wildfire
- Animals

- Wind
- Other

Other:

To be completed by the laboratory
Time of reception in laboratory _____
Record sample temperature (°C) _____
The sample meets with the criteria for reception
Yes or No

Signature or official stamp of the laboratory

_____
Signature of the sampler

ANNEX 13

EXAMPLE SAMPLE TRANSPORT PROTOCOL

A13.1 INTRODUCTION

It is important that samples are transported to the laboratory within a defined time (where appropriate, e.g. for bacteriology), under the correct conditions and in a safe manner. This example protocol gives information on how this may be achieved. Most information is given on the transport of bivalve and water samples for bacteriological analyses, where the use of coolboxes is required.

While general information is given on the transport of other samples, specific information on required transport time and conditions should be obtained from the laboratory to which the samples are to be submitted.

A13.2 TRAINING

Staff preparing samples for transport within the bivalve mollusc sanitation programme should be trained in the relevant procedures for the packing of samples and the transport of the coolboxes or other packages to the laboratory.

Where another agency or body undertakes the sample transport by formal agreement (including under contract) the responsible authority should verify that the staff of that agency or body have been properly trained. Periodic audits should be undertaken to ensure that the required procedures are being followed.

A13.2.1 *Health and Safety*

Leaking samples may pose a risk to those handling them. In addition, the outsides of containers may be contaminated with the sampled material or, in the case of bivalves, the surrounding water. There may therefore be a risk of infection from faecal contamination or, for samples of trade discharges or unknown effluents, a risk of other effects due to the presence of chemical or radiological contaminants. Disposable gloves should therefore be worn when packing samples for transport. The outside of sample containers should be dried before packing. Sample containers should also be checked for leakage in order to protect other people from contamination during transport and receipt. Leaking samples should not be packed for transport.

A13.2.2 *Communication with laborator(y/ies)*

The receiving laboratory(ies) should be made aware of the number and type of packages that are being sent, the number and type of samples being submitted, and the expected day/time of arrival. Any specific submission requirements stipulated by the laboratory for the type of samples and analyses to be performed should be followed.

A13.2.3 *Equipment – General*

> Heavy-duty polythene bags;

> Cable ties;

> Waterproof sample labels;

> Disposable gloves;

> Permanent marker;

> Validated cool-box (for temperature-controlled transport) or other appropriate packaging;

> Freezer packs (number for each box as per the validation) (for temperature-controlled transport);

> Newspaper or foam spacers for coolbox (for temperature-controlled transport);

> Completed sample submission forms.

A13.2.4 *Validation of coolboxes for microbiological and other temperature sensitive samples*

See Cefas (2007) for a procedure for validating cool boxes for use in the transport of samples taken in support of a bivalve mollusc sanitation programme. The temperature should reach between 0°C and 10°C within 4 hours of sample packing, and then be maintained within this range for at least 24 hours.

A13.2.5 *Coolbox packing*

Water samples should be securely closed before packing and bivalves should be double bagged, and the outer bag should be properly sealed (see Annex 10, Example Transport Protocol).

Samples should be packed in a coolbox of a type that has been subject to a formal validation procedure for sample transport: for water samples. Such a coolbox should be lightproof. The box should be packed in accordance with the procedure that has been validated. Spacer material (newspaper or foam) should be packed around the samples in such a way that cool packs do not come into direct contact with the samples. The number and total weight of bivalve samples should not exceed that for which the coolbox has been validated. Overloading may result in the samples not being adequately cooled and subsequently rejected at the laboratory.

> It is best to avoid mixing sample types (bivalves, large seawater samples, small freshwater/discharge samples) unless the specific mix of sample types, and number of samples of each type, has been included in the coolbox validation. In addition, although the sample packing procedures should minimize the risk of cross-contamination, it is best to transport discharge or other highly contaminated samples in a separate coolbox from those used for bivalve or other water samples.

> The packing arrangements should either prevent movement of samples or, preferably, should include separating material to keep individual samples apart.

> Place either a temperature recorder or a universal container filled with water between the samples (as close to the centre of the load as possible). The specific approach needs to be agreed with the receiving laboratory. If a temperature recorder is used, the laboratory will either need to download the data from the recorder or return the recorder to the sampler or monitoring programme manager for that purpose. In the case of a universal container containing water, the laboratory will need to record the temperature of the water as soon as possible after opening the coolbox.

> All microbiological samples should be sent to arrive at the laboratory in time for analyses to commence within 24 hours of collection unless:

> > Studies have shown that concentrations of the target microorganism(s) in the relevant sample types do not significantly increase or decrease under the target transport conditions over a specified longer period.

> > The coolboxes used have been subject to a validation study that shows that a temperature between 0°C and 10°C is maintained for that defined longer period.

> > Any samples received at the laboratory outside the target temperature range of 0° to 10°C (unless they reach the laboratory within four hours of sampling), or arrive in a frozen, or partially frozen, condition, should be discarded. However, samples received at the laboratory within four hours will be expected to have reduced in temperature from that at the time of sampling but not necessarily to have achieved 10°C (if the temperature was above this at time of sampling).

A13.3 SAMPLE TRANSPORT REQUIREMENTS

A13.3.1 *Frozen samples*

Samples to be transported frozen will usually be transported to an intermediate laboratory in a coolbox, frozen using an appropriate method, and then transported to the final destination in dry ice. In such cases, samples should be packed using the procedures given in Section 2.6. It should be ensured that the intermediate laboratory uses appropriate methods for freezing and onward transport. Further details on this are outside the scope of this example protocol.

A13.3.2 *Other packaging*

Samples that have been taken for analyses where temperature controlled transport is not needed may be transported in any sturdy container or packaging that is of an appropriate size and material for the purpose. The container or packaging should preferably be waterproof and capable of being securely closed to retain liquid in case one or more samples leak during transport.

Where there is a requirement to add a preservative to the samples for transport purposes, this needs to be done prior to the packing process (although there may be a requirement for the preservative to be added immediately after sampling).

The packing arrangements should either prevent movement of samples or, preferably, should include separating material to keep individual samples apart.

A13.3.3 *Sample submission forms*

The completed sample submission forms for all the samples in a coolbox or other packaging should either be included in the coolbox or packaging, or securely attached to the outside. The submission forms should be placed in a sealed polythene bag, or other waterproof protection so that they are not affected by any leakage or condensation (for forms included within the coolbox or packaging), or by rain (for forms attached externally).

A13.3.4 *Sample transport*

Transport of the samples to the laboratory may be undertaken by one of the following means:

In person by the samplers;

By post; or

By a commercial courier.

The chosen means should ensure that the samples are transported without exposure to physical shock or vibration, to meet prescribed requirements relating to transport time and conditions, and to avoid the possibility of interference with the integrity of the samples.

REFERENCES CITED

Cefas 2007. Coolbox Validation Protocol. Available at: https://www.cefas.co.uk/cefas-data-hub/food-safety/classification-and-microbiological-monitoring/england-and-wales-classification-and-monitoring/documents/cool-box-validation-protocol-pdf-120-kb/

ANNEX 14

EXAMPLE ANALYSIS OF RESULTS FROM PRIMARY FAECAL INDICATOR MONITORING

TABLE A14.1 **BIVALVE MOLLUSC FLESH MONITORING RESULTS**

COLLECTION DATE	*E. COLI* MPN/ 100g		
	SITE 2	SITE 3	SITE 4
07/07/11	80	20	20
15/07/11	20	80	<20
06/08/11	<20[1]	50	230
21/08/11	<20	<20	20
06/09/11	<20	140	<20
17/09/11	230	230	20
11/10/11	<20	<20	<20
23/10/11	20	20	<20
14/11/11	<20	20	50
23/11/11	<20	<20	170
07/12/11	<20	50	20
19/12/11	20	20	<20

Number of samples	12	12	12
Minimum	<20	<20	<20
Maximum	230	230	230
Percentage compliance with 230 *E. coli*/100 g	100	100	100
90th percentile from data[2]	185	203	212

1. <20 results were given a nominal value of 10 for the purposes of calculation (this being the half the nominal lower limit of detection).
2. Using the non-parametric approach given at the end of this document.
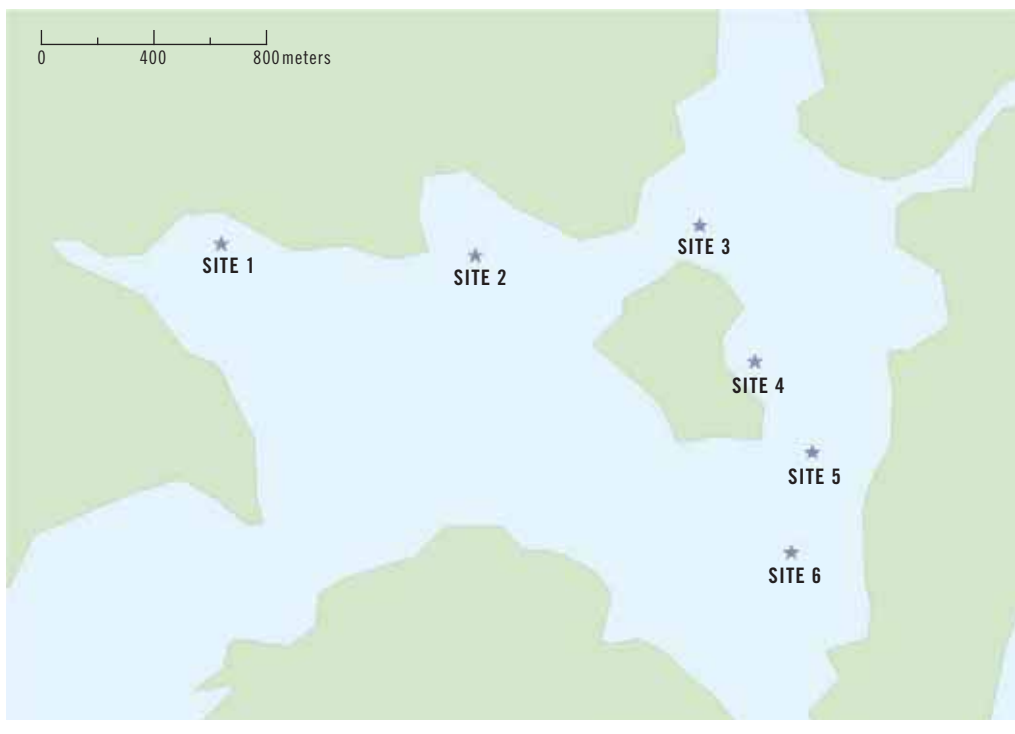
For an initial classification, pending analysis of ongoing data:

Classification based on EU criteria from 1 January 2017 (Class A – 80 percent of results ≤230 *E. coli*/100 g with no result >700 *E. coli*/100 g; Class B – 90 percent of samples ≤4 600 *E. coli*/100 g and no result >46 000 *E. coli*/100 g; Class C – no result >46 000 *E. coli*/100 g):

All sites conform to Class A

Therefore, a growing area containing all three sites would be Class A (no site is worse than Class A).

Classification based on local risk-based standards (Category I – 90th percentile ≤110 *E. coli*/100 g; Category II – 90th percentile ≤2 000 *E. coli*/100 g; Category III – 90th percentile ≤10 000 *E. coli*/100 g (Note these standards have not been produced using the approach given in the guidance document and are simply for illustrative purposes).

Site 2 = Category II

Site 3 = Category II

Site 4 = Category II

Therefore, a growing area containing all three sites would be Category II

TABLE A14.2 **WATER MONITORING RESULTS 14**

COLLECTION DATE	FAECAL COLIFORMS MPN/100 ml					
	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6
07/07/11	12	7	1	6	3	4
15/07/11	13	1	2	24	13	3
22/07/11	3	5	50	27	8	1
06/08/11	9	38	82	5	48	2
14/08/11	6	5	3	7	5	2
21/08/11	5	1	4	9	3	26
06/09/11	10	49	6	1	4	85
17/09/11	8	3	306	3	3	3
11/10/11	15	59	3	1	5	7
23/10/11	32	3	14	11	8	70
06/11/11	50	5	6	13	5	4
14/11/11	3	1	2	11	26	1
23/11/11	37	1	5	20	6	29
07/12/11	9	0[1]	3	8	6	10
19/12/11	8	14	189	11	14	5

Number of samples	15	15	15	15	15	15
Minimum	3	0	1	1	3	1
Maximum	50	59	306	27	48	85
Geometric mean	10.5	4.5	9.1	7.4	7.2	6.3
90th percentile from data[2]	42.2	53.0	236	25.2	34.8	76.0
Average $\log_{10}$	1.02	0.65	0.96	0.87	0.86	0.80
SD $\log_{10}$	0.36	0.66	0.77	0.43	0.35	0.62
NSSP estimated 90th percentile	30.3	30.6	88.4	26.4	20.3	38.4

1. A value of 0.5 was assigned for determination of the geometric mean, mean log10 and SD log10 (assuming a nominal lower limit of detection of 1 per 100 ml). A value of 1 was assigned for determination of the NSSP estimated percentile.
2. Using the non-parametric approach given at the end of this annex.

For an initial classification, pending analysis of ongoing data

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14 Beware of varying local usages when writing dates, i.e. MM/DD/YY versus DD/MM/YYYY. ISO specifies YYYY-MM-DD with lead zeroes. To prevernt confusion, it might be advisable to specify how dates should be written if the month is not spelt out.

Classification based on NSSP criteria

Criteria:

Approved: faecal coliform geometric mean shall not exceed 14 and the 90th percentile shall not exceed 49 MPN per 100 ml for a five-tube decimal dilution test

Restricted for depuration: faecal coliform geometric mean shall not exceed 88 and the 90th percentile shall not exceed 300 MPN per 100 ml for a three-tube decimal dilution test

90th percentile requirements for other MPN formats are given in the NSSP.

Sites 1, 2, 4, 5 and 6: conform to the requirements for "Approved"

Site 3: conforms to the requirements for "Restricted for depuration"

Classification based on local risk-based standards (NOTE: Example only: Category I – 90th percentile <10 faecal coliforms/100 ml; Category II – 90th percentile <200 faecal coliforms/100 ml; Category III – 90th percentile <1 000 faecal coliforms/100 ml)

Sites 1, 2, 4, 5 and 6: conform to the requirements for Category I.

Site 3: conforms to the requirements for Category II.

Therefore, a growing area containing all sites would be classified as Category II. A decision on whether to classify the sites together (on the basis of the worst case), or separately, should take into account whether different classifications for separate sites within one area can be properly enforced.

Notes

There are a large number of approaches to the estimation of 90th percentiles! Microsoft Excel uses a non-parametric approach. Other statistics packages may offer both non-parametric and parametric approaches. The parametric approach may be preferable with small data sets. However, it relies on an approximation to a normal distribution (if necessary, after transformation). This may not be valid for small data sets or even some larger ones. For example, in areas where there is marked influence of rainfall on the levels of faecal indicator bacteria, the data may follow a bimodal distribution.

The percentile rank formula is: $R = P / 100 \, (N + 1)$, where R represents the rank order of the score (the data are arranged in a list by increasing magnitude), P represents the percentile rank, and N represents the number of scores in the distribution.

For the example data, N=15 and P=90

Therefore, $R = 90/100 \, (16) = 14.4$

Therefore, the estimated 90th percentile value is the 14th value in the list plus 0.4 times the difference in value between the 14th and 15th values in the list.

The estimated 90th percentile shall be calculated by:

i.   Calculating the arithmetic mean and standard deviation of the sample result logarithms (base 10);

ii. Multiplying the standard deviation in (a) by 1.28;

iii. Adding the product from (b) to the arithmetic mean;

iv. Taking the antilog (base 10) of the results in (c) to get the estimated 90th percentile; and

v.   The MPN values that signify the upper or lower range of sensitivity of the MPN tests in the 90th percentile calculation shall be increased or decreased by one significant number.

Note that the example for local risk-based standards has not been produced using the approach given in the guidelines, and is simply for illustrative purposes.

EVENT-MANAGEMENT PLAN TEMPLATE
EXPECTED EVENTS

Each growing area should have a written management plan to cover expected events. These expected events include those that relate to conditional classifications and those that relate to any classification (conditional or otherwise) where changes in risk of one or more hazards are likely to occur during the year and where there may be a need to change the level of risk management (e.g. by means of a closure or an increased level of post-harvest processing).

HEADING	ELEMENTS TO BE ADDRESSED WHERE APPLICABLE
Scope	A list and description of each growing area to which plan applies, include classification Maps - clearly identify growing area boundary, (marine farm locations if applicable), sampling sites Period covered by the plan –when it should be reviewed.
SOURCES OF HUMAN FAECAL POLLUTION **(A SEPARATE ENTRY MAY BE REQUIRED FOR EACH SOURCE)**	
Nature of source	Identifier (e.g. name) Location (e.g. coordinates in latitude/longitude [WGS84]) Discharge location (if different from that of the asset) Permit (consent) number (if relevant) Nature of discharge (e.g. continuous, intermittent, tidally phased) Treatment level (if relevant) Any specific permit (consent) conditions and/or historical quality data (cross-refer to location of data if necessary)
SOURCES OF HUMAN FAECAL POLLUTION **(A SEPARATE ENTRY MAY BE REQUIRED FOR EACH SOURCE)**	

Criteria related to increased risk	Specify criterion or criteria (performance standard(s))
	> Related to STW or other assets;
	  > Management plans for areas affected by wastewater treatment plants, must include performance standards that adequately address;
	    > Effluent quality (microbiological, chemical, physical);
	    > Plant failures;
	    > Collection systems, pumping stations and bypasses and overflows;
	    > Design, construction and maintenance that impact on overloading or mechanical failure;
	    > Monitoring and inspection provisions;
	    > If prohibited area adjacent to outfall;
	    > Arrangements for notification of emergency (unexpected events) sewage spills will be included in the unexpected event management plan (see Annex 14);

	> Non-asset related; 　> Identification of the specific predictable event: meteorological, hydrological, salinity or other event that places the growing area in the closed status; 　> For seasonal events including boating and seasonal rainfall affecting sewage contamination, the criterion must be based on identification of the seasonal event, including estimated duration; 　> If harvest allowed for post-harvest treatment clearly identify conditions for this; 　> Map showing location of the critical measuring devices where used; 　> The predicted number of times and periods of closures, based on historical findings, that the pollution event is expected to occur in a calendar year; > Contingency plan(s); 　> What happens when critical measuring equipment used to measure performance parameters is unable to accurately and reliably measure the specified criterion.
Risk management actions to be taken when criteria are met	These may include > Closure > Reclassification > Addition post-harvest processing requirements (over and above those required by the classification)
Criteria to be met for rescinding risk management actions	Criterion or criteria to open area for example salinity, rainfall, river height, seasonal event, that reliably predict when the criteria for classification are met. Clear about time period between closure and opening. The criteria should include the time for clearance of the hazard(s) from the bivalve(s), bearing in mind that different species may clear contamination at different rates.
SOURCES OF ANIMAL FAECAL POLLUTION	
Criteria related to increased risk	Specify criterion or criteria (e.g. seasonal changes in animal numbers or grazing practices, animal markets or shows, rainfall causing land run-off, changes in abattoir operations, wild bird migration) Define how the criterion or criteria were established
Risk management actions to be taken when criteria are met	These may include Closure; Reclassification; or Additional post-harvest processing requirements (over and above those required by the classification)
SOURCES OF ANIMAL FAECAL POLLUTION	
Criteria to be met for rescinding risk management actions	Criterion or criteria to open area for example end of at risk season or reversion of agricultural practice or abattoir operation to normal conditions; salinity, rainfall, or river height, for run-off related contamination. These should reliably predict reduction in risk and that reliably predict when the criteria for classification are met. The criteria should include the time for clearance of the hazard(s) from the bivalve(s), bearing in mind that different species may clear contamination at different rates.

VIBRIOS	
Criteria related to increased risk	Specify criterion or criteria (e.g. elevated sea temperature (above what value), elevated air temperature (above what value), reduced salinity (below what value), pathogenic vibrio concentrations (seawater or bivalves) (above what value(s)) Define how the criteria were established
Risk management actions to be taken when criteria are met	These may include Closure; Temperature control during transport after harvest; or Post-harvest processing.

Criteria to be met for rescinding risk management actions	Specify criterion or criteria for rescinding the risk management actions; Define how the criterion or criteria were established; or These may simply be that the criteria related to increased risk no longer apply
BIOTOXINS	
Criteria related to increased risk	Specify criterion or criteria (e.g. season, increased phytoplankton concentration (species and concentration(s)), increased biotoxin levels in bivalve flesh) Define how the criterion or criteria were established
Risk management actions to be taken when criteria are met	These may include: Closure (the usual action) Post-harvest processing (by controlled removal of parts of the bivalve to reduce the concentration of biotoxins to acceptable levels)
Criteria to be met for rescinding risk management actions	Specify criterion or criteria for rescinding the risk management actions Define how the criterion or criteria were established These may simply be that the criteria related to increased risk no longer apply
CHEMICAL CONTAMINANTS AND RADIONUCLIDES	
Criteria related to increased risk	Specify criterion or criteria related to increased risk (e.g. seasonal increase in concentration in one or more species of bivalve mollusc, increase in contaminant loading from a known discharge, occurrence of re-suspension events (e.g. due to dredging)) Define how the criterion or criteria were established (this requires the upper level(s) deemed to be acceptable to be known or defined)
Risk management actions to be taken when criteria are met	This would normally be closure of the area while levels of one of more contaminants are above those deemed acceptable
Criteria to be met for rescinding risk management actions	Specify criterion or criteria for rescinding the risk management actions Define how the criterion or criteria were established These may simply be that the criteria related to increased risk no longer apply
COMMON ASPECTS	
Closure implementation	Be clear as to what agency is responsible for closure and opening implementation. A detailed description of how the closed status for the growing areas will be implemented. The procedures and methods for notifying regulatory agencies, industry, processors, etc. of the closure and openings. Contingency arrangements for night, weekend and absences of key personnel. Procedures to close and notify for unexpected events, e.g. sewage or chemical spills, large storm events, unexpected (high) monitoring results.
Signatory section	The event management plan developed in consultation with: the local industry; and the individuals responsible for the operation of any wastewater treatment plants, marinas, animal waste operations, etc. relevant to the plan; and any other relevant agencies involved in assessing or reporting on the criteria (performance standards) or other matters relating to the management plan.
Annexes	Contacts for relevant agencies (national, state/provincial, local authority) Contact details for harvesters, processors, packers, wholesalers, direct sales (e.g. local restaurants) Contact details for other stakeholders

ANNEX 16

EVENT-MANAGEMENT PLAN TEMPLATE
UNEXPECTED EVENTS

HEADING	ELEMENTS TO BE ADDRESSED WHERE APPLICABLE
Scope	A list and description of each growing area to which the plan applies, include classifications Maps clearly identifying growing area boundaries, (marine farm locations if applicable), sampling sites Period covered by the plan –when it should be reviewed.
Responsibilities	Identify which agencies (and, if relevant, departments/teams) are responsible for: Invoking the Unexpected Event Management Plan Undertaking investigations Undertaking sampling/testing Undertaking the risk assessment Deciding on appropriate risk management actions Undertaking associated surveillance (patrol & enforcement) activities Deciding when to rescind any risk management actions The lead agency should be clearly identified for each element. Some of these may differ depending on the hazard(s) and/or nature of the incident. If so, these should be clearly defined.
Identification of event occurrence	How will a possible unexpected event be identified? Who might provide the initial information (e.g. may be staff of the responsible authority, another agency, or the bivalve mollusc industry). These need to be considered as broadly as possible and should not be exclusive – relevant information may be obtained from a variety of sources, not all of which can be identified in a management plan.
Risk assessment process	May include: > Growing Area investigation > Contaminant Source (if not known) > Visual or other evidence of extent affected > Is this event continuing? > Epidemiological investigation > Sampling and analysis (relevant to the hazard(s)) > Information from industry on current harvest and product destination Will include: > Assessment > Outcomes > Risk management actions (see next section) > Further investigations and/or monitoring required Criteria for rescinding any closure or additional processing requirements

HEADING	ELEMENTS TO BE ADDRESSED WHERE APPLICABLE
Risk management actions	Decision tree to help decide upon relevant harvesting area constraints based on the risk assessment process Closure or additional processing requirements Patrol activities to ensure application of the harvesting area constraints Surveillance (inspection/audit activities) in dispatch centre or process establishments to ensure No product from affected area if closure instigated Required actions if additional processing required Control of traceability of product (recall if determined necessary)
Risk communication	Also see suggested annexes Collaboration between different authorities (need for Agreements or Memoranda of Understanding) Communication with harvesters (commercial and/or recreational), wholesalers (and other potential purchasers of commercial harvest)
Closure implementation	Clear what agency responsible for closure and opening implementation. A detailed description of how the closed status for the growing areas will be implemented. The procedures and methods for notifying regulatory agencies, industry, processors, etc. of the closure and openings. Contingency arrangements for night, weekend and absences of key personnel. Procedures to close and notify for unexpected events, e.g. sewage or chemical spills, large storm events, unexpected (high) monitoring results.
Follow-up	Review of the initial risk assessment (during and after the event) to determine reduction of risk from the actual hazard(s) to acceptable levels When appropriate revision or lifting of controls (including closures) Communication of outcomes
Signatory section	The event management plan developed in consultation with: the local industry; and the individuals responsible for the operation of any wastewater treatment plants involved; and any other relevant agencies involved in performance standards or other matters relating to the management plan.
Annexes	Contacts for relevant agencies (national, state/provincial, local authority) Contact details for harvesters, processors, packers, wholesalers, direct sales (e.g. local restaurants) Contact details for other stakeholders

ANNEX 17

SURVEILLANCE OF COMMERCIAL GROWING AREAS ADDITIONAL CONSIDERATIONS

A17.1 INTRODUCTION

This annex addresses surveillance activities appropriate to commercially harvested growing areas. Growing areas may be classified at different levels depending on the extent of contamination, and subsequent processing required (if any), prior to consumption. They may also be designated as closed by the responsible authority if there is a potential risk to public health due to pathogens, biotoxins, chemical contaminants or radionuclides at levels above those deemed to be acceptable. The responsible authority should establish a surveillance (patrol and enforcement) system to prevent illegal harvest in growing areas where this activity is prohibited or restricted and to ensure that product that should undergo post-harvest processing is properly dealt with. It is also necessary to prevent product from closed areas being mixed with that from open areas, or from areas requiring post-harvest processing in with that which does not.

A17.2 KEY ELEMENTS OF THE SURVEILLANCE SYSTEM

A17.2.1 *A legal basis by which the responsible authority (or another agency performing the task on behalf of the responsible authority) may conduct surveillance activities.*

A17.2.2 *Appropriate structure and functions of the body or bodies responsible for surveillance activities, including the material and human resources to perform patrol and enforcement activities.*

A17.2.3 *Identification of activities to be undertaken within the surveillance system.*

A17.2.4 *Agreements of coordination between different agencies involved in the activity, including private associations and non-governmental organizations (NGOs).*

A17.2.5 *A periodic review and report of the surveillance activities, including an assessment of their effectiveness.*

A17.3 LEGAL BASIS OF THE SYSTEM

The legal basis should allow:

1. Performing surveillance in the growing areas.

2. Putting in place agreements or memoranda of understanding with other authorities and organizations that can support the surveillance activities.

3. Establishing a system of monitoring harvesting to ensure that product is only taken from open areas and, in the case of product requiring post-harvest processing, that the product will be subjected to the appropriate post-harvest treatment.

4. Establishing prohibitions or total closures in growing areas where the product is considered a risk to public health and where no appropriate mitigation measures have been identified.

5. Application of administrative and criminal sanctions to harvesters who violate the requirements in the surveillance system.

A17.4 STRUCTURE OF THE COMPETENT AUTHORITY

The responsible authority (or another agency performing the task on behalf of the responsible authority) should be organized in such a way that allows patrol and enforcement activities to be undertaken in all growing areas (whether open, closed or prohibited). This should include having properly trained staff and appropriate equipment (e.g. boats; automobiles; aircraft; communications for coordinating patrol activities; radar surveillance systems; night scopes). The activities may be accomplished by means of a formal agreement between the authority responsible for the bivalve mollusc sanitation programme and another authority with the relevant resources and appropriately trained staff. Such authorities may include those responsible for:

> Fisheries and aquaculture;

> Protection of wild life and environment; or

> Public health.

A17.5 ACTIVITIES OF THE SURVEILLANCE SYSTEM

The spectrum of activities comprising a surveillance system may vary depending on the requirements of the sanitation programme, the legal basis for the surveillance

activities and the nature of the growing areas and types of production (e.g. aquaculture, wild harvest, harvesting method). At a minimum it should include:

Active surveillance by specialized staff in growing areas, especially in prohibited or confined areas with high levels of production of bivalve molluscs, which are attractive for people interested in obtaining product from these areas.

Control of harvesters by setting standards, issuing licenses or certificates, as well as records with specific information of growing areas where the product is obtained indicating harvest dates, the type of product and quantity obtained and special considerations (e.g., whether or not the product is obtained from a restricted area and whether or not is subjected to depuration or relay procedures or other kind of treatments).

Information on the classification status of designated growing areas, and whether they are currently open or closed, should be available to all the agencies involved in the surveillance activities and provided to producers and the general public. This system of information should ensure that harvesters and other stakeholders are aware of possible changes in the status of growing areas, especially when there is a potential risk of the presence of biotoxins.

Security measures and sanctions should be applied when illegal harvesting activities are detected. Such actions may include: seizing product, fishing gear or aquaculture equipment, application of fines and arrest of the offenders. If product is found on the market that has been harvested from prohibited or closed areas, or has not been subject to the required post-harvest treatment, the authority should ensure that the product is recalled.

The frequency of surveillance activities in the growing areas is an important consideration. There must be greater surveillance in areas that have high levels of production and those that are closed or subject to a long-term prohibition of harvest. The frequency for each growing area should be based on risk assessment (For considerations that might be applicable, see US NSSP @ .01. Control of Shellstock Growing areas. B. Patrol of Growing areas. Chapter VIII. Control of Shellfish Harvesting.

Another point to consider is the type of sanctions applied to those who violate the growing restrictions that also apply both to the authority, as well as to the type of specific activities carried out by official staff in the growing areas. Depending on the legal basis in each country, the system can give more emphasis to administrative penalties, from assurance of product and equipment to the imposition of fines or arrests of violators of the law. This aspect is also related to duties of the official authority that performs the monitoring in the growing areas. In some countries such staff can only implement the assurance of products and equipment, and they have to ask for police or naval authority support for the imposition of fines or arrests, while in some other countries, the staff can perform these actions.

Within the above indicated context, the agreements or memoranda of understanding are essential to establish agreements between authorities with different powers, so that the monitoring system becomes as complete as possible. Depending on the conditions of each country, the authority responsible for the system can establish agreements with the authority responsible for monitoring the activities of bivalve mollusc production, by issuing permits or fishing licences, as well as through the control of the environment and marine resources with the police or naval authorities. Likewise, the competent authority may establish agreements with associations of harvesters who can assist in surveillance activities, or with NGOs interested in the subject. Such agreements, or MOU, should clearly specify the scope and activities of these agencies that will support the competent authority.

A17.6 INFORMATION ON THE RESULTS OF THE SURVEILLANCE SYSTEM

A regular review of the operation and effectiveness of the surveillance system should be undertaken to confirm that it is operating effectively; the aim is to ensure compliance with the objectives.

The review should record the number and type of patrol activities, the number and type of enforcement actions, as well as data on potential system failures that have, or may have, allowed the marketing of product from closed or prohibited areas or product reaching the consumer without the required level of post-harvest processing (where this is required).

If there is evidence of major, or recurring minor, faults in the system, the surveillance system should be amended to address the problems.

A17.7 REFERENCES

Guide for the Control of Molluscan Shellfish of the National Shellfish Sanitation Program, 2015. (*see* Chapter – VIII. Control of Shellfish Harvesting)

Technical Guide of Mexican Shellfish Sanitation Program, 2009, (*See* Annex 10. – Northwest Mexico´s Patrol System and extraction control for shellstock growing areas).

Maine's Patrol System. program Element Evaluation Report 2010.

ANNEX 18

GROWING AREA REVIEW TEMPLATE

REPORT HEADER	Name and/or identifier for the area. Review period.
BACKGROUND DESCRIPTION	Classification type. Species harvested. Culture methods if applicable. Brief description of the area. Include map here or as attachment. Key stakeholders involved in programme for the area, e.g. industry, and responsible authority. Good practice to track the history of the area with key changes that have occurred since area was commissioned. Risk profile, growing area assessment or classification changes.
POLLUTION SOURCES	Give an overview of any pollution sources. Point or diffuse. Give cross-references to relevant documents. Comment on any changes to these pollution sources during the review period. Identify any new pollution sources. Include evaluation of the new or changed pollution sources and a map showing location/s. If no changes to pollution sources, report this. Comment of any unusual or emergency pollution events including sewage discharges or chemical spills impacting on the area If not dealt with in Event Management Plan section). If an area is affected by a wastewater treatment plant, provide any information on whether the performance standards for the plant were met.
HARVEST CRITERIA (IF APPLICABLE)	State harvest criteria. Comment on how the conditional management operated for the review period. Cooperation of all parties to the management plan, timeliness of reporting. Include details and dates of any calibrations carried out on critical measuring equipment used.

EVENT-MANAGEMENT PLANS	Activation of expected-event-management plans during the review period (note outcomes and any difficulties in applying the plan(s)
	Activation of unexpected-event-management plan during the review period (note outcomes and any difficulties in applying the plan)
SURVEILLANCE	If area has surveillance for any illegal harvesting, comment on compliance with any surveillance plan.
	Detail any activities where compliance action was required.
BACTERIOLOGICAL SAMPLING	Report on monitoring undertaken for the period and whether it met the relevant sampling plan(s) and protocol(s) for the period.
	Discuss any elevated results, any investigation undertaken, and any risk-management action.
	Present data analysis against standard/s used. Include a summary table of statistics for water and/or flesh for the statistical review period.
	Discuss any issues with collecting samples and laboratory analysis.
OTHER CONTAMINANT SAMPLING	e.g. Heavy metal, Toxic substances, marine biotoxins and radionuclides.
	Were the agreed sampling plan(s) and protocol(s) met?
	Comment on results and compliance with standards.
CONCLUSIONS	The relevant sampling plans and protocols compliance.
	Classification compliance.
	Harvest criteria appropriateness (where applicable)
RECOMMENDATIONS	Any required changes to
	> Growing Area boundary
	> Classification
	> Sampling plans
	> Sampling protocols
	> Harvest criteria
	> Event management plans
	> Surveillance plans
	Risk profile changes needed.
	If significant assessment work required.
ANNEXES	Monitoring data.
	Maps (here or background description section)
	Investigation reports,
	Closure notices (where appropriate)
	New growing area information, e.g. hydrographic studies,

This guidance is for completion of a review of the ongoing relevance of risk profile, growing area assessment, monitoring data, classification status and management plans. Can be annual or any other period determined necessary.

ANNEX 19

EXAMPLE ASSESSMENT OF RESULTS FROM ONGOING FAECAL INDICATOR MONITORING

FIGURE A19.1 **SAMPLING SITE LOCATIONS**

TABLE A19.1 **BIVALVE MOLLUSC FLESH MONITORING RESULTS**

COLLECTION DATE	E. COLI (MPN PER 100 G)		
	SITE 2	SITE 3	SITE 4
31 January 2012	50	20	20
15 February 2012	50	<20	130
7 March 2012	50	50	80
18 April 2012	80	80	20
16 May 2012	<20[1]	50	80
13 June 2012	20	20	20
11 July 2012	<20	130	20

COLLECTION DATE	E. COLI (MPN PER 100 G)		
	SITE 2	SITE 3	SITE 4
15 August 2012	50	50	50
12 September 2012	<20	20	<20
10 October 2012	<20	20	460
14 November 2012	50	20	110
13 December 2012	<20	170	490
16 January 2013	50	490	20
19 February 2013	<20	<20	<20
13 March 2013	20	50	50
10 April 2013	<20	130	20
15 May 2013	20	<20	<20
12 June 2013	50	40	<20
10 July 2013	<20	<20	<20
14 August 2013	<20	<20	<20
11 September 2013	<20	<20	<20
16 October 2013	50	<20	<20
13 November 2013	<20	50	140
3 December 2013	20	20	<20
15 January 2014	230	70	<20
12 February 2014	50	50	<20
12 March 2014	<20	20	<20
9 April 2014	80	20	70
7 May 2014	<20	<20	<20
4 June 2014	40	45	20
2 July 2014	<20	<20	20
6 August 2014	<20	<20	<20
3 September 2014	<20	<20	<20
1 October 2014	<20	<20	45
5 November 2014	20	170	130
3 December 2014	78	130	110
Number of samples	36	36	36
Minimum	<20	<20	<20
Maximum	230	490	490
Percentage compliance with 230 E. coli/100 g	100	97	94
90th percentile from data[2]	79	142	133

1. results <20 were given a nominal value of 10 for the purposes of calculation (this being half the nominal lower limit of detection).

2. Using the non-parametric approach given at the end of this document.

For *a three-year classification review period*

Classification based on EU criteria from 1 January 2017 (Class A – 80 percent of results ≤230 *E. coli*/100 g, with no result >700 *E. coli*/100 g; Class B – 90 percent of samples ≤4 600 *E. coli*/100 g; with no result >46 000 *E. coli*/100 g; Class C – no result >46 000 *E. coli*/100 g):

Site 2 = Class A

Site 3 = Class A

Site 4 = Class A

Therefore, a growing area containing all three sites would be Class A (no site is worse than Class A).

Classification based on local risk-based standards (Category I 90th percentile ≤110 *E. coli*/100 g; Category II 90th percentile ≤2 000 *E. coli*/100 g; Category III 90th percentile ≤10 000 *E. coli*/100 g.

[NOTE: these standards have not been produced using the approach given in the guidance document and are simply for illustrative purposes.]

Site 2 = Category I

Site 3 = Category II

Site 4 = Category II

Therefore, a growing area containing all three sites would be Category II (based on the worst classification). A decision on whether to classify the sites together (based on the worst case), or separately, should consider whether different classifications for separate sites within one area can be properly enforced.

TABLE A19.2 **WATER MONITORING RESULTS**

COLLECTION DATE	FAECAL COLIFORMS MPN/100 ML					
	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6
31 January 2012	5	4	43	4	30	2
15 February 2012	6	12	16	1	21	8
7 March 2012	2	1	5	15	16	3
18 April 2012	23	11	1	2	1	16
16 May 2012	9	23	4	1	7	5
13 June 2012	9	7	52	28	2	4
11 July 2012	36	1	7	3	4	7
15 August 2012	12	51	25	3	11	10
12 September 2012	4	10	22	14	52	6
10 October 2012	2	7	88	0[1]	12	9
14 November 2012	32	18	40	16	13	6

COLLECTION DATE	FAECAL COLIFORMS MPN/100 ML					
	SITE 1	SITE 2	SITE 3	SITE 4	SITE 5	SITE 6
13 December 2012	16	28	20	41	1	22
16 January 2013	3	4	7	10	12	19
19 February 2013	29	50	14	2	62	1
13 March 2013	3	4	0	8	3	13
10 April 2013	14	14	12	22	16	44
15 May 2013	3	15	10	2	6	17
12 June 2013	11	3	2	2	33	4
10 July 2013	11	8	7	16	6	2
14 August 2013	2	1	4	6	2	1
11 September 2013	83	1	53	1	19	32
16 October 2013	2	1	13	14	7	1
13 November 2013	2	16	4	8	5	6
3 December 2013	2	14	3	74	7	5
15 January 2014	10	1	3	1	1	16
12 February 2014	20	4	5	15	3	7
12 March 2014	3	3	15	40	50	9
9 April 2014	37	4	4	1	1	4
7 May 2014	10	3	1	10	30	4
4 June 2014	2	1	3	23	9	6
14 July 2014	6	4	3	27	14	29
12 August 2014	1	1	15	13	6	5
8 September 2014	7	5	16	33	4	13
11 October 2014	10	1	55	3	7	7
9 November 2014	14	11	4	14	4	7
12 December 2014	76	18	3	20	1	43
Number of samples	36	36	36	36	36	36
Minimum	1	1	0	0	1	1
Maximum	83	51	88	74	62	44
Geometric mean	7.7	5.2	8.2	7.0	7.2	7.1
90th percentile from data[2]	36.3	24.5	52.3	35.1	38.1	29.9
Mean log10	0.88	0.72	0.91	0.84	0.86	0.85
SD log10	0.49	0.53	0.54	0.57	0.51	0.42
NSSP estimated 90th percentile	32.6	24.6	38.5	36.7	32.9	24.8

1. A value of 0.5 was assigned for determination of the geometric mean, mean log10 and SD log10 (assuming a nominal lower limit of detection of 1/100 ml). A value of 1 was assigned for determination of the NSSP estimated percentile.
2. Using the non-parametric approach given at the end of this annex.

FOR A THREE-YEAR CLASSIFICATION PERIOD

Classification based on NSSP criteria

Criteria

Approved: faecal coliform geometric mean shall not exceed 14, and the 90th percentile shall not exceed 49 MPN per 100 ml for a five-tube decimal dilution test.

Restricted for depuration: faecal coliform geometric mean shall not exceed 88 and the 90th percentile shall not exceed 300 MPN per 100 ml for a three-tube decimal dilution test.

90th percentile requirements for other MPN formats are given in the NSSP.

All sites: conform to the requirements for Approved status.

Classification based on local risk-based standards (Example only):

> Category I – 90th percentile ≤10 faecal coliforms/100 ml;

> Category II – 90th percentile ≤200 faecal coliforms/100 ml;

> Category III – 90th percentile ≤1 000 faecal coliforms/100 ml).

All sites: conform to the requirements for Category I.

Notes on 90th percentiles

There are many approaches to the estimation of 90th percentiles! Microsoft Excel uses a non-parametric approach. Other statistics packages may offer both non-parametric and parametric approaches. The parametric approach may be preferable with small data sets. However, it relies on an approximation to a normal distribution (if necessary, after transformation). This may not be valid for some data sets. For example, in areas where there is marked influence of rainfall on the levels of faecal indicator bacteria, the data may follow a bimodal distribution.

The percentile rank formula is: R = P / 100 (N + 1). R represents the rank order of the score (the data are arranged in a LIST by increasing magnitude). P represents the percentile rank. N represents the number of scores in the distribution.

For the example data, N=36 and P=90

R = 90/100 (37) = 33.3

Therefore, the estimated 90th percentile value is the 33rd value in the list plus 0.3 times the difference in value between the 33rd and 34th values in the list.

The estimated 90th percentile shall be calculated by:

i. Calculating the arithmetic mean and standard deviation of the sample result logarithms (base 10);

ii. Multiplying the standard deviation in (a) by 1.28;

iii. Adding the product from (b) to the arithmetic mean;

iv. Taking the antilog (base 10) of the results in (c) to get the estimated 90th percentile; and

v. The MPN values that signify the upper or lower range of sensitivity of the MPN tests in the 90th percentile calculation shall be increased or decreased by one significant number.

Analysis of Results from Ongoing Faecal Indicator Monitoring for a Remote Area
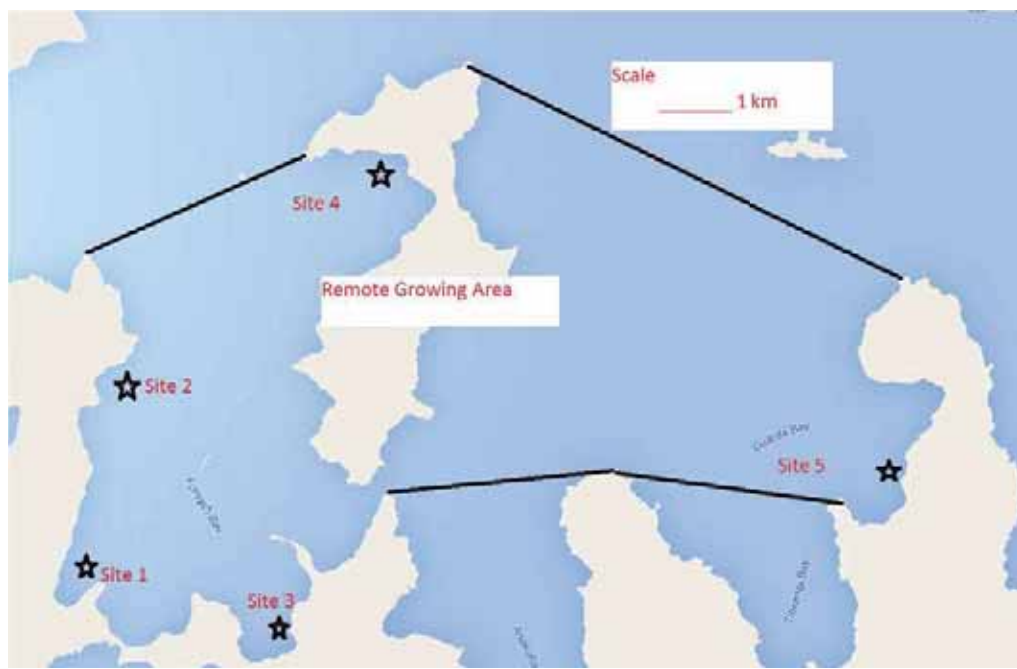
FIGUREA19.2 **SAMPLING SITE LOCATIONS**

TABLE A19.3 **BIVALVE FLESH AND WATER DATA**

DATE	FAECAL COLIFORM MPN/100ml					*E. COLI* MPN/100g	
	SEAWATER SITE 1	SEAWATER SITE 2	SEAWATER SITE 3	SEAWATER SITE 4	SEAWATER SITE 5	FLESH SITE1	FLESH SITE 5
16/04/2012	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
18/06/2012	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
13/08/2012	<2.0	<2.0	2.0	<2.0	<2.0	130	40
8/10/2012	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
11/12/2012	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20

DATE	FAECAL COLIFORM MPN/100ml					*E. COLI* MPN/100g	
	SEAWATER SITE 1	SEAWATER SITE 2	SEAWATER SITE 3	SEAWATER SITE 4	SEAWATER SITE 5	FLESH SITE1	FLESH SITE 5
11/02/2013	<2.0	<2.0	<2.0	<2.0	<2.0	<20	20
3/04/2013	<2.0	<2.0	<2.0	<2.0	<2.0	130	<20
13/05/2013	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
8/07/2013	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
9/09/2013	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
18/11/2013	<2.0	<2.0	<2.0	<2.0	<2.0	<20	20
13/01/2014	<2.0	<2.0	<2.0	<2.0	<2.0	<20	20
10/03/2014	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
12/05/2014	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
14/07/2014	<2.0	<2.0	2.0	<2.0	<2.0	20	<20
8/09/2014	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
10/11/2014	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
12/01/2015	<2.0	<2.0	<2.0	<2.0	<2.0	<20	20
9/03/2015	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20

Number of samples	19	19	19	19	19	19	19
Minimum	<2.0	<2.0	<2.0	<2.0	<2.0	<20	<20
Maximum	<2.0	<2.0	2.0	<2.0	<2.0	130	40
%>14 Faecal coliform MPN/100ml	0	0	0	0	0		
%>230 *E. coli* MPN/100g						0	0

For a three-year classification review period - Flesh

Classification based on EU criteria from 1 January 2017 (Class A 80 percent of results ≤230 *E. coli*/100 g, with no result >700 *E. coli*/100 g; Class B 90 percent of samples ≤4 600 *E. coli*/100 g and no result >46 000 *E. coli*/100 g; Class C no result >46 000 *E. coli*/100 g):

Site 1 = Class A

Site 5= Class A

Therefore, a growing area containing all two sites would be Class A.

Classification based on local risk-based standards (Category I 90th percentile ≤110 *E. coli*/100 g; Category II 90th percentile ≤2 000 *E. coli*/100 g; Category III 90th percentile ≤10 000 *E. coli*/100 g).

Site 1 = Category I

Site 5 = Category I

For a three-year classification period – Water

Classification based on NSSP criteria

Criteria:

> Approved status: faecal coliform geometric mean shall not exceed 14 and the 90th percentile shall not exceed 49 MPN per 100 ml for a five-tube decimal dilution test; and

> All sites: conform to the requirements for Approved status.

Classification based on local risk-based standards (Example only: Category I – 90th percentile ≤10 faecal coliforms/100 ml; Category II – 90th percentile ≤200 faecal coliforms/100 ml; Category III – 90th percentile ≤1 000 faecal coliforms/100 ml).

All sites: conform to the requirements for Category I.

GLOSSARY

ACCEPTED/ACCEPTABLE/APPROVED Accepted by the official agency having jurisdiction.

BIVALVE MOLLUSCS Aquatic species, such as oysters, mussels and clams, that can survive for extended periods out of water and can be traded for human consumption as live animals in shell or as product shucked and/or frozen, and generally consumed raw or partially cooked.

GROWING AREAS All brackish and marine areas approved for the production or harvesting of bivalve molluscs either by natural growth or by aquaculture destined for human consumption. The growing areas may be approved as production or harvesting areas for bivalve molluscs for direct consumption, or they may be approved as production or harvesting areas for bivalve molluscs for depuration, relaying or other processing.

GROWING AREA ASSESSMENT The actions undertaken to ensure that public health risks are properly managed and that the requirements of the growing Areas sanitation programme are complied with. It involves the production and implementation of plans to deal with expected and unexpected events, patrol of the growing area and enforcement of requirements relating to closed areas and the destination and processing of harvested product from open areas.

GROWING AREA RISK PROFILE A periodic written re-evaluation of the risk profile, growing area assessment, sampling, management and surveillance plans together with an assessment of monitoring data. The review determines whether the classification status and/or plans need to be revised.

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

LIVE BIVALVE MOLLUSCS Bivalve molluscs that are alive at the time of harvest. Live bivalves can proceed for further processing or for raw consumption.

LOCAL In relation to legislation or administration, that relating to part of a country. This may be as a result of autonomous powers, those delegated from the national government or as a result of a different tier (layer) of authority at the local level.

MICROBIOLOGICAL CONTAMINATION The presence, introduction, reintroduction, growth and/or survival of pathogens of public health concern

POST-HARVEST PROCESSING Any process which has been authorized by the responsible authority and has been validated as reducing the levels of a hazard to a level deemed to be acceptable.

RAW BIVALVES Bivalve molluscs that have been shucked and/or frozen.

REGIONAL In relation to legislation or administration, that relating to two or more countries in the same part of the world. Examples concerning food safety include the European Union food hygiene regulations and the Australia/New Zealand collaboration on food standards.

RESPONSIBLE AUTHORITY The official body with ultimate responsibility for implementation of the bivalve mollusc sanitation programme.

RISK A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

RISK ANALYSIS A process consisting of three components: risk assessment, risk management and risk communication.

RISK PROFILE A scientifically based process consisting of the following steps:
(i) hazard identification,
(ii) hazard characterization,
(iii) exposure assessment, and
(iv) risk characterization.

WGS84 World Geodetic System 1984, a geodetic coordinate system typically used for GPS positioning references.

WILD HARVEST The collection of bivalve molluscs of marketable size that develop in the natural environment without human intervention.

TECHNICAL GUIDANCE
FOR THE DEVELOPMENT OF THE GROWING AREA ASPECTS OF BIVALVE MOLLUSC SANITATION PROGRAMMES

International trade has been the main driving factor for the rapid growth of the bivalve mollusc production industry during the last six decades, growing from nearly one million tonnes in 1950 to 16.1 million tonnes in 2015. In recognition of the extensive trade of this commodity the Codex Alimentarius Commission has developed a Standard for Live and Raw Bivalve Molluscs as well as guidance in the Codex Code of Practice for Fish and Fishery Products on the steps needed to be taken at all stages of food chain in order to produce a product that meets the Codex Standard. However, to facilitate implementation of the Codex guidance, countries identified the need for more information on how to implement Codex guidance in their specific context and specifically how to establish and monitor a bivalve mollusc growing area.

This FAO/WHO Technical Guidance for the Development of the Growing Area Aspects of Bivalve Mollusc Sanitation Programmes aims to address that need. The focus of the guidance is the primary production of molluscs for consumption as live or raw bivalves and in particular how to manage microbiological hazards at this stage. Acknowledging that managing chemical hazards, toxin phytoplankton and biotoxins also presents big challenges, reference has also been provided to relevant Codex standards and other international guidance.

The guidance was developed from a technical and scientific perspective and using a risk based approach. It has been driven by the intent and experience of existing programmes, rather than the details of these programmes and in line with the requirements of the Codex Code of Practice. The guidance is primarily aimed at the authorities responsible for the development, implementation and application of a bivalve mollusc sanitation programme, while highlighting the collaboration and agreements required between different partners including local authorities, regulatory agencies and laboratories to implement such a programme.