Safe Management of Shellfish and Harvest Waters

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Safe Management of Shellfish and Harvest Waters

Edited by
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Preface

This publication presents the contributions made, conclusions reached and the consensus statement agreed upon at a workshop on safe management of shellfish and harvest waters held 30 November–2 December 2004 in Kuala Lumpur, Malaysia. The workshop was organised by the Water, Sanitation, Hygiene and Health Unit of the World Health Organization, in cooperation with the US Environmental Protection Agency, Office of Research and Development. This publication is one in a series of expert workshops, reports and monographs on Emerging Issues in Water and Infectious Disease managed by the two organizations. Other titles in the series include:

- Heterotrophic Plate-Counts and Drinking-Water Supply
- H_2S Method for Detection of Faecal Contamination
- Water Recreation and Disease
- Toxic Cyanobacteria in Water
- Waterborne Zoonoses: Identification, Causes and Controls
- Pathogenic Mycobacteria in Water
- *Legionella* and the Prevention of Legionellosis.

All publications are available from the World Health Organization website (www.int/water_sanitation_health/) or from the International Water Association (www.iwapublishing.com).

Invaluable support for the workshop was provided by the University of Malaya. A total of 22 experts from 10 different countries representing a wide range of academic disciplines, ranging from clinical and aquatic microbiology, hygiene and public health, food safety, risk assessment, epidemiology to bivalve shellfishery management attended the workshop. Meeting participants jointly examined the issues of sewage contamination of bivalve shellfish and their harvest waters. The meeting did not address biotoxins or naturally-occurring microbial contamination of shellfish.

At the workshop participants were asked to produce key technical inputs on the range of issues affecting the sewage contamination of bivalve shellfish and their harvest waters and the resultant infectious diseases. The participants were

also tasked with determining whether the existing management and control measures could, if necessary, be improved.

The global bivalve shellfish industry is a multimillion dollar industry, varying from highly commercial organizations in countries such as New Zealand to small scale, locally organized and artisanal collections in many other countries. In the latter case there are often no established methods of safeguarding the health of consumers.

This publication reflects the technical inputs made to the Kuala Lumpur workshop, associated deliberations at the workshop which may have amended those inputs and the revisions made to those amended inputs at the suggestion of the expert technical reviewers, to whom the editors are extremely grateful. A small number of additional contributions were commissioned after the Kuala Lumpur workshop from experts in additional fields and with varying perspectives to ensure the comprehensive and topical nature of the monograph – and these too were subject to external expert technical review.

This publication aims to provide relevant guidance to the appropriate agencies and stakeholders in the bivalve shellfish industry in an effort to ensure that the risks to health from consumption of shellfish associated with possible sewage contamination are minimized.

We earnestly hope that practitioners in the field will find this topical and exhaustive coverage of the subject of value to them as they strive to ensure that human health is adequately protected. We also hope that this publication will facilitate a new approach to the management of bivalve shellfish and harvest waters so that shellfish consumption is as safe and as risk-free as possible.

Acknowledgements

The World Health Organization wishes to express its appreciation to all those whose efforts made the production of this monograph possible. An international group of experts met in Kuala Lumpa, Malaysia and from that meeting provided the material for the book and undertook a process of mutual review. While authorship of individual chapters is noted below, the quality of the volume as a whole is due in large part to the review and comments provided by many individuals. Intellectual input and review by the following individuals is gratefully acknowledged:

Larry Wymer, National Exposure Research Laboratory, US Environmental Protection Agency, Cincinnati, Ohio, United States.

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This publication is dedicated to the memory of Professor Gareth Rees who died on 31 October 2008. Gareth was a committed and highly-respected microbiologist with an international reputation who worked tirelessly to complete this book. Gareth's larger than life contribution to the field of public and environmental health will be sorely missed.

1

Expert consensus

G. Rees, J. Bartram and D. Kay

1.1 CONTEXT OF THE WORKSHOP

Bivalve shellfish are filter-feeding organisms. They can concentrate microbial pollutants in marine waters including pathogenic species capable of producing disease outbreaks in consuming populations. Control of this disease risk requires integrated management of the water environment used for shellfish growing and harvesting together with post-harvest product processing which might involve depuration and/or heat treatment where appropriate. Perhaps uniquely, therefore, sustainable utilization of this food resource requires continued excellence in the quality of 'natural' harvesting waters as well as appropriate management interventions designed to correct any short-term deteriorations in environmental quality. All centres of human population produce the microbial pollutants impacting on shellfish compliance with food quality standards and also contribute the pathogens which can generate disease outbreaks. Sustainable shellfish management, therefore, presents a complex challenge of integrated

environmental management encompassing both effluent streams and receiving water quality, together with related food processing and regulation, to achieve end-product quality for consumer protection.

Harvesting shellfish on a global scale is increasing. From a series of regional concentrations, the industry has increased to a total production of 12 million tonnes in 2002, equivalent to 9.4% of the total seafood market, with exports totalling $1.4 billion in 2002. Although, in terms of global trade dollars, this is a relatively small industry, it presents disproportionate health risks because shellfish are often eaten raw or only lightly cooked.

Levels of wild source exploitation for commercial use have remained fairly constant over recent years with the increase in harvested product coming from a growth in aquaculture which comprises 84% of the total bivalve market (2002 figures).

The major global market for shellfish is Asia. The People's Republic of China is responsible for 68% of global production. Import/export of shellfish usually takes place within regional limits. For example, the bulk of live bivalve commerce in Europe is between members of the European Union (EU). China, China (Province of Taiwan), Japan, Malaysia and Thailand are key shellfish trade partners which present the potential for transboundary transport of pathogens.

Food safety is the primary issue in bivalve shellfish trade. The nature of the end product and the associated risks are significant as outlined in chapter 3 of this volume. These are compelling justifications to ensure that bivalve shellfish products are properly tracked through the food chain especially where they cross national borders. Whilst the commercial trade can be regulated relatively easily, there are issues with the 'casual' trade which characterizes this product, including its quantification and the undoubted existence of illegal harvesting. The reliability of trade statistics may be sound, but the quantum of casual exploitation is probably impossible to define.

Global experts met at a workshop held in Kuala Lumpur, Malaysia to:

- identify infectious disease risks associated with the consumption of contaminated bivalve shellfish;
- assess water quality management approaches that may reduce the risk of infectious disease; and
- examine and suggest strategies to reduce the risk from pathogens derived from human and/or animal excreta.

The workshop set out to provide guidance to health agencies, water quality and shellfish regulatory agencies and other stakeholders worldwide in recognition of

existing and potential future infectious disease problems associated with the consumption of contaminated bivalve shellfish. The efficacy of current practices in protecting human health was assessed and the need for the deployment of new approaches evaluated.

In delimiting the scope of the workshop, initial discussions centred on which shellfish and which contaminants to consider. The workshop elected to maintain an exclusive focus on bivalve shellfish – effectively filter-feeding shellfish predisposed to transmit bacterial and viral pathogens. Throughout this volume, where reference may be made, on occasion, to naturally-occurring pathogens and biotoxins, the reader will be referred to sources of authoritative information.

The workshop also addressed contaminant sources and means of transmission to bivalve shellfish, where possible identifying options to interrupt the cycle. Transmission routes were identified from land- or water-derived contamination (fresh or sea) of harvested products (including harvest for subsistence, recreational, non-market or local sale, or commercial harvest). For the purposes of this publication, post-harvest issues are considered the domain of food safety and post-infection issues the domain of health care and treatment, thus, the focus is specifically on water management aspects and strategies.

1.2 PUBLIC HEALTH FACTORS

Shellfish have been a source of food for thousands of years, as indicated by shellfish middens near ancient human habitation. Human illness caused by infectious agents translated from human or animal sources through shellfish consumption has long been identified.

Minor, self-limiting complaints predominate, although more serious illness may occur in some cases. These include cholera and hepatitis A (HAV) in less developed countries and a range of infections associated with exposure of the immunocompromised. Such illness can occur in populations dependent on shellfish as a subsistence protein source, or in populations far from the point of origin through intra-regional or international trade, i.e. potentially transmitting pathogens from endemic areas to other locations. Primary prevention of the transmission of infectious disease through shellfish requires:

1. ensuring that shellfish are only collected at places and times that minimize or eliminate the likelihood of contamination with relevant pathogens, AND;
2. methods that prevent contamination of shellfish during harvesting and transport.

   OR

3. collection at places at times of potential risk, AND;
4. depuration or post-harvest processing using procedures proven to reduce risk to tolerable levels.

Using either route, the protection of public health requires active monitoring of the source waters and the end-product in order to ensure that controls are adequate.

1.3 HARVESTING AREA MANAGEMENT OPTIONS AND RESPONSES

The workshop concluded that commercial exploitation and casual, artisanal collection of bivalve shellfish need similar levels of health protection. It was also considered important that policies are in place to ensure implementation of monitoring and regulatory regimes in commercial contexts as well as provision of unambiguous information to casual collectors on likely health risks.

Management approaches must cover the spectrum of need from highly technologically (prevalent in developed countries) to intermediate technology applications (especially in less-developed countries). Management steps can be incremental and aspirational as resources sequentially become available; i.e. not a one size fits all approach. Responses to the differing threats should therefore be upgradeable.

In terms of the transmission cycle, land and/or water-based contamination sources, that may affect product quality up to harvest, were considered. Identifying probable sources of contamination, both point (such as sewage discharges) and non-point (such as septic tanks and livestock), as well as potential management responses was considered critical to success. It should be noted that post-harvest processes are outside the scope of this monograph and are not, therefore, considered.

Exploration of available management interventions included:

Site management

- positioning harvest sites remote from known contaminant sources and provision of advice to facilitate this intervention;
- planning controls, applied at the outset of harvesting, site development, to prevent adverse effects of subsequent developments on harvest areas;
- assessment and prioritization of urban sewage and agricultural waste management actions, using studies to quantify the various source of microbial pollution and devise mitigation strategies;

- monitoring for the correct sentinels in the correct locations and with the appropriate frequency, identification and dissemination of good practice, including pollution prevention and mitigation strategies;
- sewage treatment processes to control point sources; quantification of and reduction in diffuse pollution from agricultural and other sources;
- forecasting to be applied on monitoring and other data based on the likelihood of events that may compromise shellfish integrity such as rainfall;
- creation/restoration of natural buffers between contaminant source and shellfisheries, such as wetlands.

Harvesting management
- applying the most appropriate means of purifying contaminated shellfish (such as relaying and depuration).

Education and information
- tracking shellfish from outbreak back to harvest site;
- effective communication leading up to and emanating from notices to modify practices in shellfish areas (including closure and opening notices);
- educating producers/harvesters and consumers in health-related issues;
- harmonizing systems to ease or support international trade through agreed environmental and product standards; and
- development of guidelines for commercial and recreational vessels to govern disposal of on-board contamination.

1.4 SOURCE IDENTIFICATION, SANITARY SURVEYS AND PROFILING

Contamination is often first identified through end-product (i.e. shellfish flesh) and/or environmental water sampling in harvesting areas. Remediation requires the sources of this pollution to be identified and quantified followed by practical management measures designed to reduce the pollutant flux from point and diffuse sources.

Point sources of microbial pollution, traditionally associated with end-of-pipe delivery of human and/or animal effluents, are readily identifiable and attributable. Point sources of particular relevance include livestock slaughter-house and processing effluent; overflow of manure lagoons; crude and treated sewage effluent; stormwater runoff and combined sewer overflows (CSOs).

Non-point sources, i.e. the diffuse delivery of pathogens and indicator bacteria, are far less easy to identify and include contaminated freshwater inflow or coastal movement of contaminated waters; runoff from pasture or cropland; untreated or partially treated sewage spread to land and seepage from septic tanks; leachate from landfills and fly-tipping sites. In addition, there may be a potential for faecal contamination to be mobilized from contaminated sediment (by processes such as dredging, large ship propeller wake, anchor pulling and seasonal thermocline turnover).

Intermittent sources, both point and non-point, include recreational, fishing boat and other vessel waste; large ship bilge dumping; seasonal tourist concentrations, as well as livestock and wildlife migration.

Sanitary surveys and profiling (i.e. formal assessment of pollutant sources and estimates of magnitude) are invaluable, particularly for initial selection of shellfish harvest areas. Periodic updates in survey profiles should be undertaken to assess impacts of changes and impacts of potential developments. In such surveys, there must be appropriate collation of information covering sewage outfalls; CSOs; riverine inputs; livestock; wild animals; tidal factors and currents; prevailing winds; susceptibility to and frequency of severe storm events. The data collected should provide information that can lead to decisions on when to open and close sites and such decisions must be communicated in a clear and timely fashion. These data will also inform pre-emptive closure after severe storm events and the appropriate time interval after severe events for safe reopening.

1.5 MONITORING: CHALLENGES AND OPPORTUNITIES

The basic science underpinning the monitoring processes must be reappraised and evaluated, particularly exploring the relationships between water quality measures and shellfish flesh quality. Once such relationships have been identified, it may be possible to establish relationships between indicators and pathogens in flesh and water. In addition, concerted efforts to understand species differences and the environmental drivers contributing to pathogen uptake and release will also lead to better evaluation of approaches to mitigation of shellfish product contamination, such as relay and depuration.

There is widespread agreement on the need to classify growing sites, but there is less convergence on the most appropriate methods; i.e. either by water column classification (which is potentially easier and cheaper, but relies on clear

understanding of the relationship between contaminants in water and shellfish flesh) or by shellfish flesh contamination (which is more expensive, destroys product, but provides a good surrogate risk measure and is presently required by most regulators).

It is impossible to test water or flesh for all possible contaminants. It is also generally agreed that thermotolerant (faecal) coliforms are not comprehensive indicators of health risk as their presence in water does not correlate well with the presence of bacterial or viral pathogens in flesh. The workshop participants strongly advocated that thermotolerant coliforms should be replaced by *E. coli*. Investigation of alternative and potentially more representative indicators, such as F^+RNA phages, was recommended alongside *E. coli* to provide insight into risks from viral pathogens for an appropriate appraisal period. In addition, differential indicators for water quality, shellfish flesh determinations or efficacy of management procedures such as depuration should be explored. The goal should be to provide the tools for the design of acceptable standards and interventions that can be taken to better protect public health in a range of situations.

The variations between tropical and temperate situations may also require different indicators. To identify such indicators, more data on indicators appropriate to tropical countries must be collected and approaches that can be applied to developing countries explored. In developing this approach, there should be a real effort to expand and enhance the existing sparse empirical data resource on pathogen concentrations and survival in tropical and developing countries.

Real time prediction should be applied to the modelling/forecasting of cause and effects. Parameters which could drive this approach include: salinity; rainfall; changes in turbidity; stream flows; wind direction/speed; sewage overflows; relation to sewer outfalls; and CSOs riverine inputs. Worst case scenarios and impacts of likely adverse events should be established for both baseline water quality and shellfish flesh quality.

Progressing these issues should lead to the development of risk based numerical standards, as applied in recreational waters, and could exemplify further integration of good practice in water quality assessment through modern drainage-basin management and regulation as exemplified in the US Clean Water Act and the EU Water Framework Directive.

The expert workshop participants felt strongly that a pragmatic approach to standards should be adopted – stricter standards should be in force for shellfish which are generally eaten raw as opposed to those that are eaten after cooking.

1.6 POST-CONTAMINATION PURIFICATION PROCESSES

Relaying shellfish in clean environmental waters, or in tanks of specifically treated water, produces a cleaning of pathogens from the shellfish flesh, a process commonly known as depuration. Depuration processes should be optimized with and based on technologies demonstrated to be effective in virus removal. In addition, an appropriate surrogate for viral presence (potentially F^+RNA phage) should be used to monitor the process.

Regulators should ensure that depuration is applied during the highest risk periods based on historic data used to characterize seasonal and other factors (such as those collected via sanitary survey). Some sites may require frequent depuration.

The diverse responses of different species to depuration processes must also be factored into the management regime.

1.7 COORDINATION OF AGENCIES AND COMMUNICATION

As a number of agencies are involved in regulation relevant to shellfish hygiene, it was felt that countries may wish to consider the establishment of a lead agency to ensure clear lines of responsibility and delivery of effective integration of environmental regulation measures designed to produce food hygiene outcomes with the regulation of food end-product standards. Within the matrix of organizations involved, it is typical to define roles, responsibilities and reporting lines. Coherent communication lines articulated between the competent authorities ensures that processes are not treated in isolation. As the nature of the management challenge is inherently multidisciplinary, it is essential that all appropriate actors are represented in the planning and evaluation of strategies.

Liaison with producers and industry groups is more readily undertaken than that with casual, and non-commercial harvesters. However, both categories need to be provided with the information required to make informed decisions. In extreme situations, such information may result in advisories and warnings which could, for example alert the public to consume shellfish only after applying appropriate measures to reduce risk such as cooking. In all such circumstances, interagency liaison between public health authorities, regulatory and other agencies is fundamental.

Wherever possible, stakeholders should be involved in the development and framing of legislation to ensure wide ownership and acceptance of those

regulations. It is also important to apply principles of subsidiarity. Thus, decisions should be taken at the lowest level appropriate to the issue, such as the local health authority closing shellfish harvesting waters if circumstances dictate. An important additional focus should be the development of mechanisms to discourage and counteract illegal harvesting.

Final product safety is the responsibility of those that place the product on the market. There is a clear distinction between shellfish monitoring programmes and food safety programmes which the participants in the workshop were anxious to maintain. It is important that the practicality and impact of water-based monitoring and management programmes is understood by all in the industry.

1.8 CONCLUSIONS AND RECOMMENDATIONS

The rationale for this book is the real health risks posed by shellfish consumption detailed in chapter 3. Control and reduction strategies for this risk involve improving safety of shellfish through better management of waters. This entails scientific understanding of risks, effective management tools (i.e. interventions) and management systems to enable appropriate action backed up by effective regulations developed through participatory processes and clear health based standards.

Improving the scientific basis for regulation and associated monitoring programmes is a key challenge if public health is to be protected. It is important that, where appropriate, new technologies to inform and/or create that improved scientific basis are exploited.

A sustainable shellfish industry depends on integrated management of the land and near-shore components of the coastal zone. This requires a precise understanding of land and marine environmental processes driving fluxes of faecal indicators and pathogens impacting on shellfish harvesting waters. The management agencies also require integrated working between health, environmental and food regulators and the commercial and casual exploiters of shellfish resources.

To effect this integrated exploitation paradigm, the following management and research gaps require timely attention:

1. measurements of both water and shellfish quality applying suitable indicators in various situations, including options for both low and high technology solutions;
2. developing integrated sampling strategies and evolving techniques to use measures of water quality to predict what risks shellfish might pose;
3. evaluating if, and where, composite sampling may be applied;

4. enhancing approaches to faecal source identification via established and new source tracking technologies;
5. developing risk assessment techniques and ultimately associating risk with water quality measures;
6. undertaking and applying consistent sanitary survey and profiling approaches;
7. development of models for forecasting which apply simple protocols for systems that currently exist and are locally applicable, such as rainfall and other hydro-meteorological triggers;
8. identifying and applying appropriate technologies for the remediation of land-based diffuse pollution impacting on shellfish harvesting waters; and
9. identifying processes for setting guidelines and for the integration of management agencies.

The expert meeting, supported by the evidence presented in this publication, debated the need for a framework for change, thereby devising a new approach that has universal applicability and consistency. This book outlines a series of challenging operational and research agendas now being addressed to provide sustainable management of shellfish harvesting waters and maintenance of public health. The challenges derive from the nature of the biological systems exploited and growing societal pressures causing pollution and increased exploitation. Many new scientific tools are emerging to supplement the evidence-base for regulators and operators. This development, together with the international paradigm shift in regulation towards integrated management of large scale catchment systems offers a promising suite of tools which should underpin the development and growth of the industry worldwide.

2

Bivalves: Global production and trade trends

S. Pawiro

The international trade in bivalves (shellfish) is very much regionalized. Few countries are able to penetrate distant markets outside their regions, mainly due to technical barriers such as strict regulations on imports of bivalve products in major markets. As a result, the contribution of bivalves to the total global trade in fish and fishery products was only around 2.3% of the total world export of fisheries products at approximately US$ 78.9 billion in 2005.

2.1 PRODUCTION TRENDS

The world production of bivalves i.e. oysters, clams (including cockles and arkshell), scallops and mussels, has been steadily increasing since the 1990s to reach a new record of 13.6 million metric tonnes (mt) in 2005. During the period

between 1995 and 2005, the average growth in bivalve production was approximately 5% per year. The growth was mainly attributed to two factors: the rapid growth in the aquaculture sector and a sharp increase in bivalve production in China.

Global bivalve production from aquaculture has consistently increased over the years from 7.1 million mt in 1995 to 11.9 million mt in 2005, an average increase of 6.8% annually during the period. Aquacultures contribution of bivalves to the overall bivalve production increased from 72.8% in 1993 to 87.3% in 2005. Meanwhile, the production from wild harvest has exhibited a downward trend and in fact its contribution declined from 21.5% to 12.7% during the period under review (Table 2.1).

Table 2.1 Global bivalve production by sector, 1995–2005 (in 1000 mt)

Year	Sector		Total
	Aquaculture	**Wild**	
1995	7077.1	1936.7	9013.7
1996	7188.6	1845.6	9034.2
1997	7406.0	1771.5	9177.4
1998	8013.7	1790.7	9804.5
1999	8878.9	1831.1	10 709.9
2000	9156.3	1985.0	11 141.2
2001	9920.0	2000.9	11 920.7
2002	10 419.5	2018.0	12 437.5
2003	11 217.1	2086.2	13 303.3
2004	11 650.4	1964.5	13 614.9
2005	11 861.9	1726.3	13 588.2

Source: Globefish-FAO

China became the single largest producer of bivalves with a production of 9.5 million mt in 2005, contributing almost 70% of the global harvest in that year. Japan was the second largest producer, far behind China with a production of approximately 795 000 mt (5.8%), followed by the United States of America (5.2%), South Korea (2.8%) and Thailand (2.8%). Other main bivalve producing countries are Canada, Chile, France, Italy and Spain. The bulk (±71%) of global bivalves production consists of oysters (35%) and clams (36%, including cockles and arkshell) followed by scallops (14.6%) and mussels (14.4%) (Table 2.2).

Table 2.2 World production of bivalves, by species, 1995–2005 (in 1000 mt)

Year	Oyster	Clams, cockles, arkshells	Scallops, pectens	Mussels	Total
1995	3243.1	3223.8	1690.8	1353.0	9013.7
1996	3223.8	2700.4	1811.3	1298.8	9034.3
1997	3664.8	2755.5	1802.0	1355.1	9577.4
1998	3699.9	3100.0	1429.0	1575.5	9804.4
1999	3878.6	3601.9	1564.2	1665.2	10 709.9
2000	4247.0	3431.8	1815.2	1647.2	11 141.2
2001	4403.8	3933.8	1921.9	1661.3	11 920.8
2002	4504.1	4256.5	1968.1	1708.8	12 437.5
2003	4669.2	4712.4	2023.0	1898.7	13 303.3
2004	4757.2	4944.8	1953.7	1958.7	13 614.9
2005	4781.5	4881.6	1986.2	1939.0	13 588.2

Source: Globefish-FAO

2.2 TRADE

World exports of bivalves (all product forms) reached US$ 1.82 billion in 2005, from US$ 1.41 billion in 2002, representing an increase of 29.1% during that period. In the global market more than 90% of bivalves are traded in live, fresh, frozen and dried forms, and less than 10% as canned or preserved products.

In terms of quantity, mussels dominate the global bivalve trade, accounting for approximately 57%. In terms of value, however, scallops contributed more than 45% to the total bivalve export market in 2005. Over the past 10 years, the growth in bivalve trade was mainly comprised of the growth in exports of fresh, chilled and frozen bivalves, particularly mussels, which are widely traded in international markets (Table 2.3).

The bivalve trade, as mentioned earlier, is concentrated in certain regions. The main markets for clams, cockles and arkshells are Japan and the Republic of Korea with supplies mainly from China and the Korean Peninsular. Another important market for clams is the USA with Canada as the main supplier while Spain, the most important market for clams in Europe, is supplied mainly by other European Union (EU) Member States.

2.2.1 Asia

There is an active trade in clams and cockles among south-east Asian countries particularly between Malaysia, Thailand and Singapore. Large quantities of cockles and clams from Malaysia are sold to Thailand for reprocessing (canned)

Table 2.3 Fresh, chilled and frozen bivalve exports, by species, 1995–2005 (in US$ million)

Year	Commodity				Total
	Scallops	**Mussels**	**Clams**	**Oysters**	
1995	529.3	207.9	174.6	140.9	1052.7
1996	511.2	239.6	138.6	130.2	1019.6
1997	578.9	240.3	147.0	111.8	1078.1
1998	536.1	219.1	144.8	115.8	1015.8
1999	529.0	239.7	175.8	144.0	1088.4
2000	575.9	263.6	183.5	166.9	1189.9
2001	502.4	256.9	199.8	179.0	1138.1
2002	512.1	317.1	187.2	137.7	1154.1
2003	519.8	377.3	207.0	159.1	1335.1
2004	613.8	417.9	226.6	178.7	1437.0
2005	772.6	428.8	190.1	176.2	1567.6

Source: Globefish-FAO

and to Singapore for local consumption. Meanwhile, Thailand is the largest
supplier of bivalves from the south-east Asia region, especially canned clams,
exported mainly to Canada and the USA. Singapore imported 5085 mt of other
live/fresh molluscs (mainly cockles and clams), predominantly from Malaysia –
(4738 mt) in 2006.

In general there is also growing demand for oysters and mussels in Asian
markets, particularly to satisfy the catering sector (hotel and restaurants).
Imports of mussels to major markets in south-east and far-east Asia are rising
(Table 2.4). Mussels are mainly imported from New Zealand.

Table 2.4 Imports of mussels into Asian markets (mt)

Country	2004	2005	2006
China	1528	3841	14 030
Malaysia	433	467	832
Singapore	373	390	420

Source: China Society of Fisheries, 2004; 2005; 2006; Department of Statistics, Malaysia,
2004, 2005, 2006; Singapore Trade Statistics, 2007

Japan is one of the largest markets for bivalves in Asia, and in fact the
country is the largest importer of clams, mainly from neighbouring countries like
China and the Republic of Korea. Its imports of clams in 2006 totalled 65 096 mt
and were valued at US$121.8 million, China accounting for 83% of the supply.
Overall, bivalve imports into Japan in 2006 reached 70 636 mt, worth US$160
million, with China contributing 77.6% of the share, followed by the Republic of
Korea (16.7% share) (Table 2.5).

Table 2.5 Japan: Imports of live, fresh and frozen bivalves by main suppliers, 2006 (Q = mt; V = US$1000)

Origins	Clam		Oyster		Scallop		Mussel		Total	
	Q	V	Q	V	Q	V	Q	V	Q	V
Canada	–	–	1	7	–	–	–	–	1	7
China	53 907	88 951	81	264	871	5085	–	–	54 859	94 300
The Democratic People's Republic of Korea	2836	9079	–	–	–	–	–	–	2836	9079
The Republic of Korea	7283	28 855	4542	23 249	–	–	–	–	11 825	52 104
New Zealand	–	–	258	2350	–	–	63	242	321	2592
The Russian Federation	795	1684	–	–	–	–	–	–	795	1684
Total (incl. others)	65 096	121 845	5036	27 014	891	5328	89	415	70 636	159 766

Source: Japan Fish Traders Association, 2007

China is the largest bivalve producer and also the largest market for bivalves, but it is mainly supplied from its own internal sources. The country, however, also imports high value bivalves from other countries to serve the growing demand from the catering sector. The major bivalve suppliers to China are the Republic of Korea, New Zealand, USA and Canada (predominantly mussels, clams and oysters).

Other important markets for bivalves in Asia are China, Hong Kong Special Administrative Region (SAR), China (Province of Taiwan) and Singapore. In 2006, Hong Kong (China) imported 20 000 mt of bivalves mainly from mainland China, Japan, USA and Canada while China (Province of Taiwan), an important market for oysters and scallops, imports mainly from the USA, Canada and Japan. Tables 2.6 and 2.7 how the imports of oysters and scallops respectively into Asian markets in 2004, 2005 and 2006.

Table 2.6 Imports of oysters into Asian markets (mt)

Country	2004	2005	2006
China	553	496	517
China (Hong Kong SAR)	4126	5613	5138
Malaysia	178	117	517
The Republic of Korea	84	175	517
Singapore	789	766	916

Sources: China Society of Fisheries-China, 2004, 2005, 2006; Hong Kong Census and Statistics Department; Department of Statistics, Malaysia, 2004, 2005, 2006; Korean Customs Service, 2004, 2005, 2006; Singapore Trade Statistics, 2007

Table 2.7 Imports of scallops into selected Asian markets (mt)

Country	2004	2005	2006
Malaysia	691	599	693
The Republic of Korea	4500	5266	6002
Singapore	704	941	1007

Sources: Department of Statistics, Malaysia, 2004, 2005, 2006; Korean Customs Service, 2004, 2005, 2006; Singapore Trade Statistics, 2007

2.2.2 European Union

In Europe the most important bivalve markets are France, Italy and Spain. The trade is mainly intra-regional between EU Member States with a smaller contribution from third countries. The United Kingdom and France produce oysters, and Denmark, Ireland, The Netherlands and Spain produce mussels.

Only a few third countries, such as Chile and New Zealand, are able to penetrate the EU markets. France, the largest mussel consumer in Europe, imported 41 200 mt in 2006, with almost 88% of supplies coming from fellow EU Member States.

The EU Member States, particularly France, Italy and Spain import significant amounts of scallops, clams and cockles from third countries such as Canada, various Latin American countries (particularly Argentina, Chile and Peru) and the USA. Fresh and frozen clams and cockles are imported from Morocco, Tunisia and Turkey. Canned products are mainly from south-east Asia and Chile. Table 2.8 shows the yearly mussel imports by France, by product form and by country of origin (in 1000 mt).

Table 2.8 Yearly mussel imports by France, by product form and by country of origin (in 1000 mt)

Country Fresh/chilled/live	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Greece	*	*	*	*	*	*	*	*	*	*	5.2
Ireland	4.5	4.8	4.1	6.8	7.0	13.9	9.6	9.9	8.5	6.2	5.7
Italy	*	*	*	*	*	*	*	*	1.8	3.8	4.7
The Netherlands	14.5	14.0	29.3	16.5	11.1	10.5	8.5	15.0	15.5	14.8	10.5
Spain	5.0	7.0	5.9	7.2	6.4	6.6	6.5	5.5	8.0	6.8	9.7
The United Kingdom	3.6	5.4	4.3	4.9	3.9	7.3	3.7	3.6	3.3	3.1	2.1
Others	4.6	3.5	4.8	4.0	4.6	6.4	5.4	4.5	5.6	4.9	6.6
Total	32.2	34.7	48.4	39.4	33.0	44.7	33.7	38.5	42.7	39.6	39.3
Frozen[**]											
Ireland	1.4	1.9	1.2	1.0	0.9	1.3	1.1	1.1	1.3	1.1	1.0
The Netherlands	0.6	0.5	1.9	0.3	0.1	0.1	0.1	0.0	0.0	0.0	0.1
Others	2.6	2.1	6.0	0.8	1.1	0.7	0.4	0.5	1.0	1.1	0.8
Total	4.6	4.5	9.1	2.1	2.1	2.1	1.6	1.6	2.3	2.2	1.9
Grand Total	36.8	39.2	57.5	41.5	35.1	46.8	35.3	40.1	45.0	41.8	41.2

* included under "others"; ** including dried, salted and in-brine.
Source: Globefish-FAO

2.2.3 United States

In 2006, the USA imported 26 916 mt of fresh/frozen and dried scallops mainly from Canada, China and Japan. Imports of fresh and frozen oysters and clams were supplied mainly by Canada while canned products were mostly imported from Canada, China, Thailand and Viet Nam (Table 2.9).

Table 2.9 United States bivalve imports, 2006

Products	Total (MT)	Main suppliers (%)
Oyster		
Fresh/frozen	5194	The Republic of Korea (38%) Canada (47%)
Canned	5954	The Democratic People's Republic of Korea (38%) China (60%)
Scallop(meat)		
Fresh/frozen/dried	26 916	Canada (13%) China (50%) Japan (12%)
Clams		
Fresh/frozen	4985	Canada (71%)
Canned/prepared/preserved	10 554	Canada (13.9%) China (30%) Thailand (13.9%) Viet Nam (15%)

Source: National Marine Fisheries Service, Fisheries Statistics Division, Silver Spring, MD, USA (Personal communication). More information is available at: http://www.st.nmfs.noaa.gov/st1/

2.3 TRADE ISSUES

Trade in bivalve species between developing countries and major markets has not developed as well as that for other seafood products. This is mainly because of public health concerns. Importing countries enforce strict regulations on live, fresh and frozen bivalves which many exporting developing countries are unable to meet.

Under the EU import regulations on bivalves, currently only 13 third countries are authorized to export their bivalves to the EU markets (http://circa.europa.eu/irc/sanco/vets/info/data/lists/lbm.html). From Asia, only Japan, the Republic of Korea, Thailand and Viet Nam are currently qualified to export their bivalves to the European Community. This contrasts with other general seafood products, where approximately 100 third countries and territories have been approved to export their products to the EU. Almost all major seafood producers in Asia have been approved by the EU authorities.

Similarly, for export of live, fresh and frozen bivalves to the United States market, exporting countries need to establish a memorandum of understanding (MoU) with the United States Food and Drug Administration (USFDA).

In general, exporting countries must meet the standard stated in the National Shellfish Sanitation Programme. So far only Canada, Chile, New Zealand and the Republic of Korea have signed the MoU with USFDA, providing them with access to the USA market.

Singapore, one of the main bivalve markets in the south-east Asia region, also applies stringent import inspection procedures on bivalve products which are considered to be of high health risk. Imports of bivalves must be accompanied by a health certificate from the competent authority in the country of origin and samples are collected from every consignment for laboratory tests.

2.4 CONCLUSIONS

There is a growing demand for bivalves, not only in historically developed countries, but also in developing regions such as south-east and far-east Asia. The main concerns with the bivalve industry relate to the pre-harvest stages where monitoring of biotoxins, pollution and management of production areas remain problematical, with many producing countries failing to meet the strict requirements imposed by consuming nations.

Assistance is needed in improving the pre- and post-harvest practices to produce satisfactory product quality and safety. Thus, the prospects for growing the bivalve industry in developing countries will depend on their ability to build reliable monitoring and inspection programmes and implement sustainable farming practices.

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3

Adverse health outcomes

T.K. Graczyk, K. Suresh and D. Lees

The popularity of molluscan shellfish in the diet is growing because shellfish contribute low-fat proteins, thought to enhance health (Rippey 1994; Munoz 1999; Wallace *et al.* 1999). However, concerns have been raised worldwide regarding health risks from molluscan shellfish contaminated with human pathogens of anthropogenic and agricultural origin (Feldhusen 1990; Todd *et al.* 1992; Potasman *et al.* 2002; Table 3.1). Most of the reports of outbreaks of infection have come from the United States, although there are some reports from Europe, Australia and Asia. Since the late 1800s when shellfish-related illnesses were first reported in the United States, there have been over 400 epidemics of foodborne diseases and over 14 000 gastroenteritis cases related to consumption of contaminated molluscan shellfish (Rippey 1994). In New York, USA alone from 1980 to 1994, over 85% of Norwalk-like virus (NLV) outbreaks, and all foodborne outbreaks of *Vibrio* spp. and *Plesiomonas shigellosis*, were associated with seafood consumption (Wallace *et al.* 1999). Molluscan shellfish accounted for 64% of all foodborne epidemics in which the

etiologic agent was identified ($n = 204$), and for 41% of outbreaks caused by an unknown etiologic agent ($n = 12$) (Wallace *et al.,* 1999). In the mid-1990s, over 98% of the incidence reports and 99% of the case reports of *Vibrio* spp. associated gastroenteritis and primary septicaemia were reported as due to consumption of raw oysters (Rippey 1994). Regarding non-*Vibrio* spp. associated diarrhoeal diseases, oysters and hard shell clams were identified in more than 56% and 38% of foodborne outbreaks and in 54% and 44% of foodborne disease cases respectively (Rippey 1994).

Molluscan shellfish are well-recognized vectors of human enteropathogens and marine biotoxins. Oysters are more likely than other seafood items to contain infective microorganisms because they concentrate pathogens from surrounding waters and are very often eaten raw (Rippey 1994; Wallace *et al.* 1999). In the United States, 8% of approximately 33 million foodborne illnesses annually have been linked to the consumption of raw oysters (Altekruse *et al.* 1999). Clams, mussels, cockles and scallops are less of a public health concern because they are usually consumed cooked or steamed, which significantly alters the infectivity of potential pathogens (Rippey 1994).

Foodborne illnesses related to consumption of molluscan shellfish have been classified into three categories based on the origin of the etiologic agent:

- human enteropathogens associated with raw sewage disposal, waste-water effluents, and overboard disposal of faeces;
- infectious agents or marine biotoxins indigenous to coastal waters, such as autochthonous bacteria, *Vibrio* spp.; and
- post-harvest contamination (Rippey 1994).

3.1 BACTERIAL AND VIRAL GASTROENTERITIS RELATED TO WASTEWATER AND SEWAGE DISPOSAL

In the mid 1990s in the United States, over 75% of gastroenteritis outbreaks and over 79% of gastroenteritis cases associated with the consumption of shellfish contaminated by sewage or wastewater-derived pathogens were due to an unknown etiologic agent (Rippey 1994). Gastroenteritis of unknown etiology occur more frequently in late spring and late fall, roughly coinciding with periods of the most intense shellfish feeding and associated pathogen bio-accumulation (Rippey 1994). It is believed that approximately 50% of foodborne outbreaks of unknown etiology related to the consumption of raw

Table 3.1 Reports of *Cryptosporidium* spp. in molluscan shellfish intended for human consumption (chronological order)

Shellfish species and reference	Geographic location	Identification level; detection techniques	Comments
Crassostrea virginica (Fayer *et al.* 1998)	Choptank, Severn, Miles, Wye, Potomac, and Wicomico Rivers; Chesapeake Bay, USA.	*Cryptosporidium parvum*; IFA, mouse bioassay.	Infectious oocysts in hemolymph and gills, most infected oysters at a site near a large cattle farm.
C. virginica (Fayer *et al.* 1999)	Fishing Bay, Tangier Sound, and Wicomico, Nanticoke, Potomac, and Patuxent Rivers; Chesapeake Bay, USA.	*C. parvum* Genotype 1 and 2; IFA, mouse bioassay, PCR, PCR-RFLP.	Oocysts detected in oysters and water, infectious oocysts in hemolymph and gills.
Dosinia exoleta, Venerupis pullastra, V. rhomboideus, Venus verrucosa, Mytilus galloprovincialis, Ostrea edulis, Ruditapes philippinarum (Freire-Santos *et al.* 2000).	Galicia, Spain; Italy; England.	*Cryptosporidium* spp.; malachite green, safranine, methylene blue, carbol-fuchsine, auramine-rhodamine, IFA.	Depuration ineffective for *Cryptosporidium* spp. positive relationships between faecal coliforms and *Cryptosporidium* spp. in shellfish.
M. galloprovincialis, Cerastoderma edule (Gomez-Bautista *et al.* 2000).	Galicia, Spain.	*C. parvum* Genotype 2, IFA, mouse bioassay, PCR, RFLP.	Oocysts infectious, $> 10^3$ oocysts/ mollusc, most contaminated shellfish near river banks with grazing cattle.
Mytilus edulis (Lowery *et al.* 2001)	Shores of Belfast Lough, Ireland.	*C. parvum* Genotype 1; IMS-IFA, PCR, PCR-RFLP.	Anthropogenic source(s) of contamination.
C. virginica (Fayer *et al.* 2002)	Chesapeake Bay, USA.	*Cryptosporidium* sp.; IFA	Oyster contamination coincided with rainfalls and increased stream-flow.
C. virginica (Fayer *et al.* 2003)	Atlantic coast from Maine to Florida, USA.	*C. parvum, C. hominis; C. meleagridis,* IFA, PCR, genotyping, mouse bioassay.	65% commercial harvesting sites contaminated with *Cryptosporidium* spp.
C. virginica (Gomez-Couso *et al.* 2004)	United Kingdom.	*C. parvum, C. hominis;* multiplexed nested PCR.	*C. parvum* and *C. hominis* detected in 11% of sampled bivalves.

IFA, immunofluorescent antibodies; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restricted fragment length polymorphism.

oysters, are due to viral agents (Wilson and Moore 1996). In over 93% of molluscan shellfish-associated outbreaks, the shellfish were probably contaminated at the sites from which they were harvested as opposed to post-harvest contamination (Wallace *et al.* 1999).

3.1.1 Viral gastroenteritis

The predominant viral agents implicated in molluscan shellfish outbreaks include the diverse group of NLVs (noroviruses; caliciviruses) and hepatitis A (HAV) (Rippey 1994; Munoz 1999; Wallace *et al.* 1999). Outbreaks of HAV have been reported consistently from around the world since the early 1960s (see for example, Rippey 1994; Wallace *et al.* 1999; Furuta *et al.* 2003). However, in the past two decades NLVs have been the predominant cause of viral gastroenteritis (see for example Huppatz *et al.* 2008). The principal presentation of NLV infection is an acute onset of vomiting or diarrhoea, or both. The illness is generally mild and self-limiting, with symptoms lasting 12 to 48 hours. In seven separate outbreaks of NLV gastroenteritis, the median incubation period was 31 hours (range: 2 to 69 hours) and the median duration of illness 48 hours (range: 10 hours to 7 days) (Anonymous 1993; 1996). The attack rate was 91% among people who consumed more than five dozen oysters, and 46% among those who consumed less than one dozen (Anonymous 1993). A study in the United States showed that the attack rate in multi-state outbreaks due to consumption of raw or steamed oysters contaminated with NLV ranged from 43% to 100% (Anonymous 1994). The attack rates of NLV gastroenteritis due to overboard disposal of faeces into the oyster bed, with subsequent harvesting and distribution of contaminated oysters, varied from 58% to 83% (Kohn *et al.* 1995; McDonnell *et al.* 1997).

3.1.2 Bacterial gastroenteritis

In general, the bacterial agents of shellfish-vectored illnesses represent a small portion of all outbreaks and cases, 4.0% and 3.8%, respectively (Rippey 1994). Identified bacteria listed include the causative agent of typhoid fever (*Salmonella* spp., such as *S. typhi*), *Shigella* spp., *Campylobacter* spp., *Plesiomonas shigelloides*, *Aeromonas hydrophila*, and *Escherichia coli* (Rippey 1994; Weber *et al.* 1994; Munoz 1999; Potasman *et al.* 2002). Historically, typhoid fever was a significant public health problem among consumers of raw oysters (Rippey 1999). After the deaths of several citizens in New York, USA in the early 1900s following the consumption of contaminated oysters,

outbreaks due to *S. typhi* were successfully eliminated by:

- implementation of more effective sewage treatment;
- reconstruction of storm and sewerage systems; and
- institution of national surveillance systems for infectious disease outbreaks (Rippey 1999).

The last case of oyster-related typhoid fever reported in the United States was in 1954 (Rippey 1999).

3.2 SHELLFISH-VECTORED ILLNESSES RELATED TO AUTOCHTHONOUS BACTERIA

Several *Vibrio* spp. have been identified as the causative agents of molluscan shellfish-related diseases with the severity of the disease depending on the contracted species of *Vibrio* (Blake 1983; Rippey 1994; Weber *et al.* 1994; Shapiro *et al.* 1998). These include: *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* O1 and non-O1 serotypes, *V. fluvialis*, *V. hollisae*, *V. mimicus*, *V. damsella*, *V. metschnikovii*, *V. furnissi*, and *V. alginolticus* (Blake 1983; Rippey 1994; Weber *et al.* 1994; Shapiro *et al.* 1998). *Listeria* spp. have also been detected in frozen and fresh shellfish and in coastal waters (Todd *et al.* 1992; Richards 2003).

3.2.1 *Vibrio* spp. infections

In general, gastroenteritis associated with *Vibrio* spp. is much more severe than diarrhetic diseases of viral etiology (Rippey 1994). *V. vulnificus* can cause infections resulting in fulminant primary septicaemia (often with necrotizing cutaneous lesions) associated with a high mortality rate that can reach up to 61% (Hlady *et al.* 1993; Rippey 1994; Weber *et al.* 1994; Wallace *et al.* 1999; Table 3.2). Beside diarrhoeal disease, *V. vulnificus, V. fluvialis*, *V. hollisae*, *V. mimicus*, and *V. parahaemolyticus* can cause extra-intestinal infections (cholecystis) and wound and ear infections (Blake, 1983; Shapiro *et al.* 1998, Table 3.2). The population at risk for *V. vulnificus* septicaemia include people with pre-existing conditions such as:

- liver diseases due to cirrhosis, hepatitis, or alcohol overuse;
- renal disease; certain medical conditions (e.g., diabetes, hemochromatosis, leukaemia and anaemia); and
- immunosuppressive disorders (Hlady *et al.* 1993; Rippey 1994; Weber *et al.* 1994; Wallace *et al.* 1999).

Table 3.2 Clinical syndromes and infections caused by various *Vibrio* species. Frequent +++, less common ++, and rare +

Species	Gastroenteritis	Wound infection	Ear infections	Septicaemia
V. parahaemolyticus	+++			+
V. vulnificus	+	++		++
V. cholerae O1	+++			
V. cholerae non-O1	+++	++	+	+
V. fluvialis	++			
V. hollisae	++			+
V. mimicus	++		+	
V. damsela		++		+
V. metschnikovii	+			+
V. furnissi	+			
V. alginolticus	+	++	++	+

The case–fatality ranges from 50% to 63% among this group (Hlady *et al.* 1993; Rippey 1994; Weber *et al.* 1994; Wallace *et al.* 1999); this population is at 80 times greater risk of illness and over 200 times greater risk of death (Hlady *et al.* 1993). Morbidity and mortality due to *V. cholera* O1 and non-O1 serotypes have been sporadically reported among shellfish consumers in the United States (Rippey 1994; Weber *et al.* 1994). In one outbreak, raw oysters were associated with eight cases of *V. cholera* O1 (Weber *et al.* 1994). All implicated oyster lots had been harvested from the Gulf Coast waters and shipped interstate (Weber *et al.* 1994). *V. cholera* non-O1 infections have been associated with mortality, although this serotype does not cause such severe gastroenteritis as O1 serotype (Rippey 1994; Weber *et al.* 1994). Interestingly, the use of antacids predisposes a person to foodborne *Vibrio* spp. infections by neutralizing the protective gastric acid barrier (Munoz 1999).

3.2.2 Seasonal pattern and distribution of *Vibrio* spp. infections

Case reports of *V. vulnificus* due to consumption of raw oysters show a seasonal pattern with the highest frequencies from midsummer through to late autumn (Blake 1983; Rippey 1994; Shapiro *et al.* 1998). This bacterium has been identified in oysters at the highest densities when the water temperature exceeds 15°C (Rippey 1994; Anonymous 1996; Shapiro *et al.* 1998; Wallace *et al.* 1999). *V. vulnificus* can occur in oysters legally harvested from open oyster beds and properly handled prior to their consumption in a raw form (Shapiro *et al.* 1998).

3.3 POST-HARVEST CONTAMINATION OF SHELLFISH BY BACTERIA

The bacteria involved in this type of contamination, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*, are derived from equipment, utensils and premises used for processing of molluscan shellfish, and from food handlers (Todd *et al.* 1992; Rippey 1994).

3.4 HUMAN WATERBORNE PARASITES AND MOLLUSCAN SHELLFISH

Cryptosporidium parvum, *Giardia lamblia*, *Cyclospora cayetanensis* and *Toxoplasma gondii* are human protozoan enteropathogens in which transmission is associated with water (Wolfe 1992; Ortega *et al.* 1993; Graczyk *et al.* 1997; Lindsay *et al.* 2001). *C. parvum*, *G. lamblia,* and *C. cayetanensis* infections cause gastroenteritis, which is predominantly manifested by diarrhoea (Wolfe 1992; Ortega *et al.* 1993; Graczyk *et al.* 1997d). *T. gondii* causes serious congenital complications in foetuses born to mothers infected for the first time during pregnancy. Medically, the most important is *Cryptosporidium* spp. as it significantly contributes to the mortality of people with impaired immune systems (Graczyk *et al.* 1997). Although *G. lamblia* (syn. *G. intestinalis*, *G. duodenalis*) and *C. cayetanensis* cause serious prolonged diarrheal illness in adults and children worldwide, the infections usually respond well to pharmacologic treatment (Wolfe 1992; Ortega *et al.* 1993). *C. parvum, G. lamblia* and *T. gondii* are anthropozoonotic pathogens (Wolfe 1992; Graczyk *et al.* 1997; Lindsay *et al.* 2001). All of these parasites produce a long-lasting and environmentally resistant infectious stage – *Cryptosporidium* spp. *Cyclospora* spp. and *Toxoplasma* spp. produce oocysts and *Giardia* spp. cysts, which can be transmitted via water. *Cryptosporidium* spp. oocysts pollute coastal waters via point and diffuse sources of contamination, such as wastewater discharges, leaky septic tanks, urban runoff, recreational activities, and agricultural runoff predominantly from livestock operations, namely cattle farms (Graczyk *et al.* 2000a; 2000b). Clinical infections are mainly confined to calves, which can shed up to 10^6 oocysts per gram of their faeces, and exceed 10^9 oocysts in daily output (Anderson 1981). As many as 10^6 oocysts per ml can be found in human diarrhetic faeces (Rose 1997). The infectious dose of *C. parvum* for immunosuppressed people has not been established, but it is believed that the disease can be caused by a single oocyst (Rose 1997). Mortality rates due to *C. parvum* among these individuals vary from 52% to 68% (Rose 1997).

In addition to *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma* spp. and *Cyclospora* spp., human-infectious microsporidia such as *Encephalitozoon intestinalis*, *E. hellem* and *Enterocytozoon bieneusi* are emerging anthropozoonotic pathogens that inflict considerable morbidity on healthy people and can be associated with mortality in immunosuppressed populations (Bryan and Schwartz 1999). The transmissive stages of all these parasites, oocysts, cysts and spores respectively, are resistant to environmental stressors and are therefore relatively widespread in the environment (Wolfe 1992; Rose *et al.* 1997; Kucerova-Pospisilova *et al.* 1999). *Cryptosporidium* spp. and *Giardia* spp. are very frequently transmitted via water (Wolfe 1992; Rose *et al.* 1997). Considerable evidence gathered to date also indicates the involvement of water in the epidemiology of microsporidia (Sparfel *et al.* 1997; Dowd *et al.* 1998; Cotte *et al.* 1999; Fournier *et al.* 2000).

Molluscan shellfish are suspension- or sediment-feeding organisms, which filter unicellular algae, bacteria, other microorganisms and detritus in approximately the 1–30 μm particle size range (McMahon 1991; Kennedy *et al.* 1996). The diameter of *Cryptosporidium* spp., *Cyclospora* spp. and *Toxoplasma* spp. oocysts does not exceed 6, 8 and 10 μm, respectively, while *Giardia* spp. cysts are oval and no longer than 15 μm (Wolfe 1992; Ortega *et al.* 1993; Graczyk *et al.* 1997; Lindsay *et al.* 2001). Microsporidian spores range from 1.5 to 4 μm (Graczyk *et al.* 2004). Thus, cystic stages of these parasites fall within the range of particles filtered by bivalve molluscs.

Historically, *C. parvum* oocysts of waterborne origin were first identified in the tissue of blue mussels in Ireland (Chalmers *et al.* 1997), initiating worldwide investigation of this pathogen in molluscan shellfish (Graczyk 2003a; 2003b). Since then, multiple studies have demonstrated that these filter-feeding organisms can harbour environmentally-derived protozoan parasites as a result of concentrating the recovered particles (Graczyk 2003a; 2003b).

An interesting epidemiological discovery is the identification, for the first time, of human-infectious microsporidia spores, *E. intestinalis* and *E. bieneusi*, in a molluscan shellfish, the zebra mussel (*Dreissena polymorpha*) (Graczyk *et al.* 2004). Microsporidia infect a variety of vertebrate and invertebrate hosts, and approximately 14 species have been reported to infect people (Kotler and Orenstein 1999). Of these *E. intestinalis* and *E. bieneusi* have been recorded as zoonotic, involved in the infection of domestic animals and livestock (Deplazes *et al.* 1996; Bornay-Llinares *et al.* 1998; Breitenmoser *et al.* 1999; Rinder *et al.* 2000; Buckholt *et al.* 2002; Graczyk *et al.* 2004). In humans they cause serious gastroenteritis, as well as urinary and sometimes ocular infections (Graczyk *et al.* 2004). Although the actual transmission route of this specific spore species is not known, it is quite possible that infectious spores of human or animal origin

passed to the aquatic environments via faeces or urine (Bryan and Schwartz 1999). Spores of microsporidia have been detected in a variety of surface waters (Avery and Undeen 1987), which becomes a source of human infections (Cotte *et al.* 1999). In addition, spores of *E. intestinalis* and *E. bieneusi* have been detected previously in surface waters (Sparfel *et al.* 1997; Dowd *et al.* 1998).

3.4.1 The public health threat from molluscan shellfish contaminated with *Cryptosporidium* spp.

Prior to 1992, the association between contamination derived from animal faecal wastes and the occurrence of shellfish-vectored illnesses was inconclusive (Stelma and McCabe 1992). In 1994, enterohemorrhagic *Escherichia coli* O157 became a major concern (Rippey 1994). Beginning in 1998, multiple studies worldwide indicated that molluscan shellfish intended for human consumption can be contaminated with *Cryptosporidium* spp. (Table 3.1). So far there has been no reported outbreak (or case) of foodborne cryptosporidiosis linked to consumption of raw oysters (Potasman *et al.* 2002); however,

- a large proportion of foodborne infections linked to oyster consumption are in the category of an unknown etiologic agent (Anonymous 1996);
- epidemiology of enteric infections (cryptosporidiosis) indicates an association with consumption of raw shellfish; and
- it is believed that the true incidence of shellfish-vectored gastroenteritis is considerably underestimated (Potasman *et al.* 2002).
- there is no mandatory requirement for reporting of gastroenteritis of an unspecified nature and physicians and health departments may not forward case reports to authorities (Rippey 1994; Wallace *et al.* 1999).

3.4.2 Methods used for identification of human protozoan parasites in molluscan shellfish

Methods for identification of human protozoan parasites in the tissue of molluscan shellfish include:

- immunofluorescent antibodies (IFA) alone or in combination with immunomagnetic separation (IMS);
- polymerase chain reaction (PCR) alone or combined with Restricted Fragment Length Polymorphism (RFLP) for genotyping;
- multiplexed nested PCR;
- fluorescent *in situ* hybridization (FISH).

Infectivity of the parasites recovered from the shellfish is usually assessed by mouse bioassays (Graczyk 2003a; 2003b).

Because *Cryptosporidium* spp., *Giardia* spp. and microsporidia can infect a variety of non-human hosts (Wolfe 1992; Graczyk *et al.* 1997; Kotler and Orenstein 1999), identification of human–infectious species is a challenge. Another challenge is the determination of the viability of these environmentally recovered pathogens, as they may be non-viable and thus have no epidemiological significance. Although molecular methods are very sensitive and specific they do not assess infectivity of the pathogens recovered from shellfish. Both challenges are met by the FISH technique. FISH employs fluorescently labeled oligonucleotide probes targeted to species-specific sequences of 18S rRNA, and therefore identification of pathogens is species-specific (Graczyk *et al.* 2004). Also, as rRNA has a short half-life and is only present in numerous copies in viable organisms, FISH allows for differentiation between viable and non-viable pathogens (Jenkins *et al.* 2003; Graczyk *et al.* 2004). FISH has been combined with direct IFA against the wall antigens of *Cryptosporidium* spp. and *Giardia* spp. and this approach has been successful for detection of *C. parvum* and *G. lamblia* in shellfish samples (Graczyk *et al.* 2004). For identification of viable pathogens such as *C. parvum*, *G. lamblia* or human-infective microsporidia, FISH is advantageous over other techniques including PCR because it allows simultaneous species-specific identification, visualization and viability assessment of single pathogen cells. Such resolution is either unavailable, or extremely impractical with any other technique. For example, using recently developed highly sensitive RT-PCR, the lowest number of *C. parvum* oocysts which can be assessed for viability is 10^3 (Jenkins *et al.* 2000).

3.5 BIAS IN REPORTING OF MOLLUSCAN SHELLFISH-VECTORED ILLNESSES

The data reported in medical literature most likely represents only a small portion of actual gastroenteritis cases, as the true incidence of shellfish-vectored illnesses is believed to be considerably underestimated (Hauschild and Bryan 1980; Mead *et al.* 1999). There are several reasons for such under-reporting, including:

- a lack of mandatory requirements for reporting of gastroenteritis of an unspecified nature because gastroenteritis is not a reportable illness (Rippey 1994; Wallace *et al.* 1999);
- many cases of gastroenteritis are mild and self-limiting and hence do not require treatment by a physician (Rippey 1994; Wallace *et al.* 1999);

- not all outbreaks are recognized or reported and sporadic cases of foodborne illnesses are not detected by the existing foodborne disease surveillance system (Wallace *et al.* 1999);
- it is difficult to epidemiologically ascribe a diarrhetic disease outbreak to a specific food item, particularly when small numbers of people are showing symptoms (Rippey 1994; Archer and Kvenberg 1995; Wallace *et al.* 1999);
- for some infectious agents, symptoms may not become apparent immediately, but instead appear long after the implicated food items have been consumed or discarded (Rippey 1994; Wallace *et al.* 1999); and
- the accuracy of the tagging system is not perfect (Rippey 1994).

3.6 METHODS OF SHELLFISH SANITIZATION

Molluscan shellfish destined for human consumption can be subjected to processing such as cooking/heating (pasteurization), relaying, depuration, irradiation, ozonation and high hydrostatic pressure in order to remove or inactivate potential microbiological contaminants. These methods have been applied predominantly to purge or inactivate bacterial and viral agents (Richards 2003), and the published information on their efficiency for protozoan parasites is limited. Gomez-Couso *et al.* (2003a) demonstrated that depuration was ineffective in removing *C. parvum* oocysts from mussels, oysters, clams and cockles harvested from contaminated waters. Gomez-Couso *et al.* (2003b) also demonstrated that molluscan shellfish contaminated with *C. parvum* oocysts can spread this contamination within the commercial depuration plants to other aquatic organisms processed in such facilities.

3.7 WHY ARE ILLNESSES CAUSED BY SHELLFISH CONSUMPTION NOT ANTICIPATED TO DECLINE IN THE FUTURE?

There are several reasons why shellfish-vectored outbreaks and related cases of gastroenteritis are not projected to decline.

- The faecal coliform count, which is the main standard indicator for waterborne faecal contamination, is not reliable in determining the quality of water at shellfish harvesting sites (Rippey 1994; Anonymous 1996; Wilson and Moore 1996). The transmissive stages of enteropathogens can persist in aquatic environments for greater lengths of time than the

enteric indicator bacteria (Graczyk and Schwab 2000; Richards 2003). Thus, waters considered to be "safe" based on the faecal coliform standards can be contaminated by enteropathogens of anthropogenic or human origin (Rippey 1994; Anonymous 1996; Wallace *et al.* 1999; Graczyk and Schwab 2000). Monitoring techniques are discussed in more detail in chapter 7 of this publication.

- Animal operations such as individual farms, industrial animal production facilities, or concentrated animal feeding operations located near shores can generate enormous surface runoff, particularly under adverse weather conditions, and can cause water pollution (Freire-Santos *et al.* 2000; Gomez-Bautista *et al.* 2000).

- Deficiencies at sewage treatment plants such as volume limitations related to designed capacity of a plant under adverse weather conditions (e.g., heavy rainfall), allow the discharge of large amounts of unprocessed waste waters. In addition, the periodic breakdown in particle removal, or inadequate disinfection can deliver human enteropathogens into shellfish-harvested waters (Rippey 1994).

- Transmissive stages of human enteropathogens are resistant to environmental degradation (including heat, sunlight, temperature fluctuations) and may even remain infectious after exposure to chemical water treatment processes such as chlorination (McDonnell *et al.* 1997; Graczyk and Schwab 2000). These pathogens can still be infectious even after the oyster meat has been processed (McDonnell *et al.* 1997; Graczyk and Schwab 2000) and are also only slowly depurated (removed) from molluscan shellfish tissue (Graczyk and Schwab 2000).

- Increased faecal pollution determined by the faecal coliform counts has decreased the total area of coastal habitats approved for harvesting of molluscan shellfish (particularly oysters) for human consumption in some areas. Thus, there is evidence that large and very productive areas have been closed, resulting in illegal harvesting of oysters from unapproved or closed, but profitable waters (Rippey 1994). Such criminal activity unavoidably affects public health when contaminated shellfish enter the market (Rippey 1994).

- Improper post-harvest handling and transportation of molluscan shellfish (such as inappropriate temperature control) affects oysters directed for consumption in a raw form (Rippey 1994). Holding of oysters at temperatures greater than 4°C in transit or in the market place can contribute to multiplication of bacterial enteropathogens (Rippey 1994).

- Many shellfish-related outbreaks have more than one contributing factor (Wallace *et al.* 1999). For example, contaminated ingredients added to

raw or lightly cooked molluscs have also been reported as contributing factors for foodborne infections (Wallace *et al.* 1999). Development of new molecular techniques which can be applied to a wide variety of food items has dramatically increased the sensitivity and specificity of detection of human enteric parasites in the tissue of molluscan shellfish (see citations in Table 3.1).

3.8 CONCLUSIONS

Molluscan shellfish can efficiently filter, retain and accumulate in their tissues environmentally derived human enteropathogens without affecting their infectivity. Therefore, such shellfish can cause human foodborne illnesses if consumed raw or after inadequate processing. Information derived from epidemiologic investigations and surveillance systems indicates an upward trend in foodborne illnesses in some areas linked with consumption of molluscan shellfish.

Worldwide, the majority of outbreaks have been linked to oysters followed by clams and mussels, and most of the reports originate from the United States, followed by Europe, Asia and Australia (Potasman *et al.* 2003). HAV virus caused the largest shellfish associated outbreak, but NLV caliciviruses have caused the highest number of outbreaks (Potasman *et al.* 2003). *Vibrio* species are the most common bacterial pathogens in shellfish. Foodborne illnesses following consumption of molluscan shellfish continue to occur throughout the world despite the fact that:

(1) testing of waters for faecal coliforms from which oysters are harvested for human consumption often demonstrates that the water quality meets the criteria of national standards or guidelines;
(2) oysters harvested from such waters are considered as "safe" with regards to faecal pollution;
(3) sanitation procedures at oyster-harvesting facilities meet national or local standards; and
(4) in most instances, neither confirmed evidence of improper handling or processing of outbreak-implicated oysters nor the environmental source(s) of pollution are detected.

These facts indicate that the monitoring of water for faecal coliforms at molluscan shellfish-harvesting sites may not be sufficient for indicating the presence of human enteropathogens of anthropogenic or agricultural origin.

Reducing the number of outbreaks and cases of foodborne diseases due to bivalve molluscs will require the coordinated efforts of different agencies with

responsibility for water quality assessment, shellfish harvesting and processing, disease surveillance and consumer education (Rippey 1994; Anonymous 1996; Wallace *et al.* 1999). It may be useful to reduce or eliminate economic incentives for illegal harvesting of shellfish from unapproved or prohibited waters that result in contaminated shellfish reaching the marketplace (Rippey 1994).

Prevention of foodborne gastroenteritis caused by consumption of molluscan shellfish requires consumer education to ensure thorough cooking of shellfish (Rippey 1994; Wallace *et al.* 1999; Freire-Santos *et al.* 2000; Graczyk and Schwab 2000). Education should be particularly focused on populations that are predisposed to serious illness after consumption of contaminated shellfish, including people with pre-existing liver or kidney diseases, diabetes and suppressed immune systems (Rippey 1994; Wallace *et al.* 1999). Several factors may impede consumer education campaigns about the risk of raw shellfish consumption (Altekruse *et al.* 1999). For example, point-of-sale warning signs may not reach vulnerable populations (Altekruse *et al.* 1999). This occurred in New York, USA where victims of a foodborne outbreak of *V. vulnificus* infection who suffered from liver diseases, did not know that they should avoid consumption of raw oysters (Wallace *et al.* 1999).

It may be useful to issue guidelines for sanitization of molluscan shellfish to reduce pathogen counts in molluscan shellfish or inactivate potential pathogens, via cold shock, heat shock, pasteurization, relaying, depuration or irradiation (Altekruse *et al.* 1999).

Continued surveillance for outbreaks and cases of gastroenteritis associated with consumption of raw shellfish are needed to assess the efficacy of current practices designed to prevent human illnesses. Public health officials should consider consumption of raw shellfish as a possible source of infection during the evaluation of a gastroenteritis outbreak (Rippey 1994; Wallace *et al.* 1999; Graczyk and Schwab 2000).

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4

Driving forces and risk management

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Bivalve shellfish are widely distributed and have been exploited as a source of food for thousands of years, archaeologists regularly finding shellfish middens near ancient human habitation. This role as a food source has also led to human illness caused by infectious agents transmitted from human or animal sources through shellfish consumption (see chapter 3). Such illness can occur in populations dependent on shellfish as a subsistence protein source, or in populations far from the point of origin consuming this product as a result of intra-regional or international trade. Whilst most recognized illnesses transmitted this way are seen as nuisances, they can be deadly, especially to humans with previously compromised immune systems.

Shellfish are filter-feeders and as a result they can concentrate pathogenic microorganisms from the environment, even from waters that meet regulatory standards. Outbreaks of diseases in humans due to contaminated shellfish have been reported in both developed and developing countries. The microbial agents associated with shellfish-related illnesses can be of bacterial, viral, or protozoan

origin. Such a diversity of microbial agents represents a considerable challenge from a monitoring standpoint and for scientists trying to develop comprehensive exposure prediction models. Whilst illnesses associated with eating contaminated shellfish are well recognized (including gastroenteritis, hepatitis and toxin-related poisoning), in most cases the actual etiological agents are unknown. However, it is assumed that viruses are responsible for the majority of cases of unknown etiology. Viruses have also been responsible for sizeable outbreaks. Indeed, the largest outbreak reported so far is the epidemic of hepatitis A (HAV) in China in 1988 in which 288 000 people were affected after eating raw or improperly cooked clams (Butt *et al.* 2004a, 2004b).

Outbreaks of gastroenteritis due to bacterial pathogens can also be significant, although in many cases these cannot be attributed to faecal pollution. For example, outbreaks associated with shellfish contaminated with *Vibrio parahaemolyticus* have been reported from Japan, USA and other countries. In Japan, between 1996 and 1998, there were 1710 incidents and 24 373 cases of *V. parahaemolyticus* (FAO/WHO MRA 02/02) and reported food poisoning cases due to this organism outnumbered those due to *Salmonella* spp. Between 1997 and 1998, more than 700 cases of illness due to *V. parahaemolyticus*, the majority of those related to raw oyster consumption, were reported from the USA. Emergence of cases due to the pandemic serotype of *V. parahaemolyticus* O3:K6 in the United States has led to greater attention on the pathogenic microorganisms that may be transmitted to humans through shellfish.

Monitoring of shellfish flesh or shellfish waters for the presence of pathogens and indicator organisms are two of the strategies available for public health protection (see chapter 6). Each of these strategies can be challenging. In chapter 13, Busby explains how the 2004 amendments to the regulations in New Zealand suggest monitoring both growing waters and shellfish flesh.

4.1 SCOPE OF THE MONOGRAPH

From a public health perspective, food safety is the overall goal and there are two distinct areas where interventions to this end can take place, either pre- or post-harvest. Pre-harvest, water quality management is the focus whereas post-harvest quality management depends on the nature of the particular processes undertaken. This monograph focuses exclusively on water quality management and pre-harvest processes. In effect, post-harvest processes are the remit of food safety and post-infection issues belong in the realm of health care and treatment.

Further, in the context of water quality management, the primary risk factors are pathogens (human, animal, naturally occurring), toxic algae and chemical contaminants. This volume concentrates on infectious disease risks posed by

microbial contaminants rather than toxins. It is acknowledged that management of environmental bacterial pathogens (of non-faecal origin like *Vibrio* spp.) as well as algal toxins is extremely difficult as their levels are impacted by environmental factors as well as nutrient fluxes. There is undoubtedly more scope for management interventions in the case of human and/or animal derived pathogens which form the focus of the monograph.

Before considering the package of interventions available, three key questions need to be answered: Which contaminants? Which shellfish? Which parts of the transmission sequence? The answer to the first question has been discussed in the preceding paragraphs – bacterial and viral contaminants. The answer to the second question is similarly succinct, the shellfish of interest are filter-feeding bivalve shellfish predisposed to transmit bacterial and viral pathogens. The answer to the third question is more complex as there are many stages where interventions could occur. For the purposes of this monograph, the focus on the transmission sequence is from land- or water-based contamination of water (fresh or sea) to harvest of contaminated product (including harvest for subsistence, recreational, non-market or local sale, or commercial harvest).

4.2 MANAGEMENT OPTIONS AND INTERVENTIONS

4.2.1 Sources of potential sewage contamination

As is generally the case, point sources of contamination are readily identifiable and include treated water effluent; stormwater runoff; combined sewer overflow (CSO); livestock slaughterhouse and processing effluent; overflow from manure lagoons. Coastal regions are often highly populated and commonly have further seasonal influxes. Wastewater treatment becomes an issue, particularly in times of any seasonal population highs. The presence, quantity, survival and infectivity of human pathogens in the wastewater and agricultural run-off that may pollute shellfish waters is of key importance.

As noted in chapter 1, non-point sources are more difficult to both identify and quantify because of their diffuse delivery mechanisms and because they include contaminated freshwater inflow or coastwise movement of contaminated waters; runoff from pasture or cropland; untreated sewage; seepage from septic tanks; seepage from landfills; and release from contaminated sediment that may be disturbed in a variety of ways. In addition, intermittent sources of faecal material include recreational or fishing boat waste; large ship bilge dumping; seasonal tourist concentration; livestock or wildlife migration.

The key goal is to focus on identifying contaminant sources and means of transmission to shellfish and then selecting options to interrupt the cycles.

In some cases, identification of primary sources can be performed using sanitary surveys. Understanding of landscape and land use is also critical. In other cases, more sophisticated microbial source tracking methods are needed to more accurately determine the relative importance of different faecal pollution sources (USEPA, 2005 and fully discussed in chapter 5).

4.2.2 Current management responses

One of the most widely used management approaches is the regular micro-biological monitoring of shellfish harvesting areas/shellfish tissues and classifying the areas. Shellfish harvested from areas with <14 faecal coliforms (geometric mean) per 100 ml water are designated by United States Food and Drug Administration (USFDA) as "approved", while in the European Union (EU), samples of shellfish with <230 *E. coli* or <300 faecal coliforms per 100 g of flesh are classified as category A. Shellfish from these areas can be used for human consumption without further processing. Shellfish harvested from areas with higher faecal coliform or *E. coli* counts are to be used only after depuration, relaying or heat processing (see chapter 9). In both the EU and the USA, commercial depuration is subject to legal control and purified shellfish are required to comply with end product standard for shellfish sold live. However, human volunteer studies in Australia (Grohmann *et al.* 1981) and natural outbreak studies in United Kingdom and USA (Lees 2000) suggest that depuration may fail to eliminate enteric viruses from contaminated shellfish and that compliance with *E. coli* or faecal coliform standard does not guarantee absence of viruses. This is further confirmed by a number of studies that show that viruses are eliminated at a much slower rate compared to faecal coliforms (Schwab *et al.* 1998).

In the case of bacterial pathogens like *V. parahaemolyticus* the pathogens are generally present at low levels ($<10^2$/g) and the infective dose is $>10^5$ (Sanyal and Sen 1974). Therefore, one of the management options to prevent human illness is to prevent bacterial growth in oysters by rapid chilling of the oysters immediately after harvest.

Cooking shellfish is another alternative for public health protection. Millard *et al.* (1987) demonstrated that the HAV virus could be inactivated by more than 4 $\log_{10}$ infectious units by raising the internal temperature of shellfish (cockle) meats to 85–90°C for one minute. However, data for other viruses, including norovirus is lacking. Since norovirus is believed to be responsible for the most common gastrointestinal illness associated with shellfish consumption, heat inactivation data for this virus would be useful. The major problem in getting this data is that noroviruses cannot be cultivated. Studies of Slomka and

Appleton (1998) indicate that feline calicivirus is inactivated more readily than hepatitis virus. Though adequate cooking will reduce the risk of human infection with viruses, a study in the USA of a multi-state outbreak of norovirus gastroenteritis associated with oysters, suggested that home or restaurant cooking offered little or no protection (McDonnell *et al.* 1997).

4.2.3 Source protection

Management practices that remediate the source of contaminated water are of great value to the ultimate goal of preventing shellfish contamination. Such measures include locating sources of contamination away from water bodies. Waste material can then be collected and treated prior to entry into fresh or coastal waters. Wherever possible, dedicated efforts should be made to treat all sewage discharged to water bodies, to redesign sewage transport systems to eliminate CSOs and to redesign or re-site manure lagoons to prevent catastrophic overflows. In addition, development of new contaminating sources should be discouraged at sites that could threaten the integrity of shellfish waters.

Natural buffers or filters (such as wetlands, mangroves, settling ponds, riparian or littoral filters) could be created/restored between contaminant sources and at-risk waters. Such a strategy should include controls over wastewater or bilge dumping from commercial and recreational vessels in the vicinity of shellfish waters.

It is essential that management efforts between watersheds and/or river basins and coastal zones are co-ordinated. This will require communication between local, state, and federal agencies and defining regulatory standards that can achieve both water and shellfishing goals. Additionally, management approaches will benefit from incorporating multidisciplinary perspectives (such as hydrology, ecology and microbiology). This philosophy should extend to nation states with adjacent waters.

4.2.4 Source management

Once the raw water has been contaminated, management options may switch to managing the water, increasing the focus on and speed of decay of potential pathogens. Methods to achieve this could include enhancing dilution by increasing mixing; promoting adsorption to sediment; introducing bacterio-phages after known contamination events; introducing non-contaminating flocculants; and installation of artificial shellfish beds/towers prohibited from harvest to clean the water. In reality, such interventions are largely speculative and untried and have varying likelihood of success in a real-world situation.

4.2.5 Shellfish management

As the end-point of the primary production process, the shellfish themselves provide the most accessible and effective management options. First amongst those options is defining no-take zones, by creating a general coastal survey or zone map to identify areas not likely to be suitable for shellfish harvest (such as areas not naturally supporting shellfish; areas with insufficient nutrient and water flow; and waters close to densely populated areas, ports or coastal industries). Second in the hierarchy of responses is to conduct an in-depth sanitary survey of current and likely future harvest sites, ensuring that such areas are sited away from known contaminant sources. This could lead to opening and closing of areas based on monitoring and management of contributing factors (particularly rainfall) highlighted in the sanitary survey (see chapter 8).

Once sites have been identified for future exploitation or historically exist, the application of monitoring tools is a logical next step. Thus, where possible, waters should be monitored for multiple contaminants in conjunction with shellfish flesh monitoring for those actual contaminants detected by water quality monitoring. This would inform harvest, relaying or depuration activities until the shellfish flesh no longer shows contaminant or any such contamination is at some acceptable level.

The final and most effective option is to control consumption, although for casual collections that is virtually impossible.

4.3 RISK MANAGEMENT

4.3.1 Risk management components

Monitoring and surveillance activities are fundamental to understanding the human health risks associated with contaminated shellfish. An additional tool available to risk managers is quantitative risk analysis (QRA) which is applied in a four step process as described by McBride (2004), namely hazard assessment, exposure assessment, dose–response analysis and risk characterization. The concentration of pathogens (hazard assessment) and the nature and degree of exposure (exposure assessment) varies according to the individual, location and situation. A risk profile can be calculated based on the distribution of these variables. QRA relies on information from other studies, including monitoring and surveillance and scientific literature. The risk characterization can be used to modify and refine management efforts, thereby leading to more effective public health practice. Details of the methodology can be found in several texts, including Haas *et al.* (1999), Haas and Eisenberg (2001).

As indicated earlier, the manager's armoury is considerably enhanced by site selection tools, including sanitary survey, modelling and monitoring in an attempt to reduce risk at source. Identification of key parameters in site selection processes that could improve safety considerations is an important function.

4.3.2 Monitoring milieu

One of the current imponderables in the shellfish safety debate is whether it is better to monitor shellfish flesh or water column quality. Added to that is the lack of confidence in currently accepted indicators to adequately reflect risk to human health whether derived from shellfish flesh or the water column. The challenge to scientists is to understand the impacts of water quality on the quality of shellfish flesh. As a minimum this would entail calibration of indicator levels between the two systems. The resolution of these issues would considerably enhance the confidence of risk managers in the depuration and relaying processes.

In terms of the risk management process, having access to real-time data on water quality and/or shellfish quality to inform decision making in a timely fashion would be a huge advance. Source tracking becomes increasingly important – attributing pathogens to human or animal sources, particularly in a timely fashion, will provide enhanced quality of information on the likely hazard posed by a specific type of contamination (see chapter 5).

As an example, multiple strains of norovirus have been implicated in foodborne outbreaks, and agricultural inputs can contribute significantly to norovirus levels. This is an upcoming area for discussion in the context of shellfish and water. We are only just beginning to understand human and animal viruses after huge investment in time and resources and we are relatively unaware of many animal viruses. As an illustration, there are many animal noroviruses recognised but these have been little studied to date. We should not make assumptions – animal sources may be important reservoirs of new pathogenic variants that could be transmissible through shellfish consumption. The issue of animal–human transfers also should be scoped in this context.

4.3.3 The commercial imperative

Very little focus appears to be on the responsibility for the additional cost burden associated with an enhanced set of safety mechanisms around shellfish consumption. It can be safely assumed that the primary responsibility for additional costs will lie with the producers in the first instance. Inevitably these costs will subsequently assimilated by consumers. It is not realistic to assume

that additional costs will be a positive driver for a safer shellfish harvest management process – rather the reverse.

This cost factor leads in to the response of the food industry to any changes that may be prefaced in this monograph. Undoubtedly a key driver is that of trade in bivalves as a food commodity (as discussed by Pawiro in chapter 2) – a driver that often appears to compete with health protection. Risk managers have a responsibility to focus on the health protection issues rather than the trade dimension. Casual gathering is an extremely difficult area to control and one that certainly complicates public health controls. The tensions between commercial exploitation and casual exploitation need to be explored and the inherent difficulties in controlling casual collecting must be recognised. If necessary, a range of models need to be constructed to accommodate all options – one size does not fit all.

4.3.4 The challenge

It is important to establish a framework to work within when analysing a complex issue such as this – fundamental controls must be delineated and the surrounding framework established from there. This will, in turn, allow scientists to attempt to resolve the key questions: Are current risk management strategies effective? Are they so ineffective that there is need for a paradigm shift or could they be made more effective by incremental modifications? These are matters that we will return to in chapter 17 following their exploration within the subsequent sections.

4.4 OPTIONS FOR THE FUTURE

At the end of this monograph we expect to be able to understand more fully whether there is an underlying requirement for a new management and regulatory framework for the safe management of shellfish and harvest waters. This brief chapter has outlined the driving forces behind the current need for a regulatory framework, but has also hinted at shortcomings in existing frameworks. It should be noted that both socio-political, as well as economical factors, will play important roles. In all considerations, however, public health should be regarded as the principal goal.

4.4.1 Primary conflicts

It is generally accepted that there is a need to classify growing sites, but little agreement on what methods to employ: either by water column classification (easier, cheaper, but implies a clear understanding of the relationship between

contaminants in water and shellfish quality which is often lacking); or by shellfish flesh contamination (more expensive, destroys product, but measures actual risk, and is often required by importing countries). Adding to this is the use of different standards by different countries which can impact on international trading.

It is impossible to test harvest waters or shellfish flesh for all possible contaminants and so indicators are sought. However, authorities generally agree that faecal coliforms and *E. coli* are not effective indicators because their presence does not correlate with pathogen presence in flesh and their presence does not correlate well with viral pathogens in either the water environment or the shellfish flesh.

4.4.2 Requirements of control mechanisms

The exploitation of shellfish as a food source by man requires societal controls to limit infectious diseases caused by pathogens to which shellfish are exposed. The usual note of caution should be applied when considering casual gathering and their limited response to regulatory and control mechanisms.

The minimum requirement regarding the appropriate combination of management options is that they establish a system to identify and manage sources of contamination. That system must be accompanied by the development of regimes to monitor contaminants in water prior to harvest and in shellfish flesh prior to consumption. Evaluation of implemented management practices could be introduced to assess pollution control effectiveness and to further confirm the identification of the primary faecal pollution source(s).

Once shellfish have been contaminated, there must be access to clearly described protocols to purify them, undoubtedly based on existing relaying and depuration processes. In terms of surveillance, it is also essential to have well established methods available so that the regulator can track shellfish from outbreak back to harvest site.

None of these processes are easy and will require a concerted campaign to raise awareness amongst producers/harvesters and consumers. That will in turn more readily enable controls on shellfish harvest to be more easily enforceable if any measures are built on a sound evidence-base. Additionally, in an ideal world there would also be movement towards harmonised systems to ease or support international trade.

4.4.3 Requirements of science

To ensure that the regulatory and control bodies have good science upon which to base legislation poses significant challenges to the scientific community.

To accomplish that, science needs to provide means to resolve fundamental issues. Thus we must be able to track contaminants back through system to source, which implies accurate techniques to detect and measure contaminants in water and/or flesh. These detection techniques will be particularly valuable if they are developed with a view to providing data in "real time". Emerging developments in microarray, nanotechnologies and bioinformatics will provide the opportunity to simultaneously determine the presence of indicators, pathogens, and source identifiers, increasing the accuracy of risk assessment models and hence usefulness of environmental monitoring (Santo Domingo *et al.* 2007). Until then, the imponderable of identifying suitable proxies for contaminants, indicators, must be resolved if the contaminant of concern cannot itself be readily identified or measured.

Regulators must be armed with the tools to identify events or conditions that could change waste/contaminant system interactions with the receiving waters and so could affect open/closed harvest practices. The obvious example here is the effect of rainfall: Can we make valid predictions on the likely effect that known amounts of rainfall within a catchment may have on contaminant levels in shellfish beds? In this context, science is challenged to develop models to predict system response to a contamination event and thus, for example, to proactively close harvest areas.

Obviously these technologies, as they are developed, will be equally applicable to the evaluation of methods of shellfish contamination mitigation, i.e. relay and depuration.

4.5 CONCLUSIONS

It has been clearly established that the harvest of shellfish for human consumption is accompanied by significant risk to human health from, amongst other things, pathogenic microorganisms associated with faecal waste. What is obvious is that health impacts and monitoring of these impacts is relatively underdeveloped. Different countries or regional alliances look to different regulatory tools to cope with the risks, such as the EU Water Framework Directive and the US Clean Water Act and the concept of riparian retirement as applied in New Zealand. Earlier chapters in the monograph (see chapter 2) discussed the increase of world trade in bivalve shellfish. That in turn demands more and better collaborations nationally and internationally, which will consequentially ease trade negotiations.

Challenges exist for regulators and scientists, not least to determine the relative threat posed by different organisms, thereby enabling priorities to be set and defining a hierarchy of the really important organisms to govern responses.

This can, in turn, inform the wider public health picture – possibly linking water based recreation issues with those of shellfish and shellfish harvest waters. It may be that, for example, we can learn valuable lessons for shellfish water management from recreational water management as discussed by Kay *et al.* in chapter 15. What is obvious is that a consensus exists for change to better reflect the actual risks and thus to protect the consumer.

Subsequent chapters in the monograph will address the issues that have been raised in this brief chapter. This will lead to a rounded appraisal and, if appropriate, a framework for change, based on a well reasoned approach will be recommended in the final chapter.

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5

Identification of primary sources of faecal pollution

J.W. Santo Domingo and T.A. Edge

Waterborne pathogens from faecal pollution continue to be major contributors to outbreaks of infectious disease in many areas around the world. While developed countries have made much progress in municipal wastewater treatment and management of agricultural wastes, faecal contamination of source waters for drinking, recreation and rearing of shellfish still contributes to outbreaks of infectious disease. The burden of gastrointestinal disease in developing countries, much of which is waterborne, is already enormous and estimated at greater than 26 billion cases per year (Payment and Riley 2002). It is anticipated that emerging waterborne pathogens, many of faecal origin, will likely pose additional challenges in the future (WHO 2003; WHO 2004). It is vitally important for managers of shellfish programs to understand the primary sources of faecal pollution in shellfish waters so that a pollution prevention

approach, or if necessary, a targeted remediation effort can be taken to reduce faecal contamination and the likelihood of outbreaks of shellfish-borne disease.

5.1 DEFINITION

Microbial source tracking (MST) is an emerging field that seeks to predict the source of microbial contamination in the environment. The field has been developing rapidly from a growing need to determine the source(s) of faecal contamination in aquatic environments. It is based on the assumption that, given the appropriate method and faecal source identifier, the source of faecal pollution can be detected (US EPA 2005). Source identifiers are the genotypic or phenotypic traits used as targets to detect host specific microbial populations in environmental waters. In typical MST applications, microorganisms from aquatic environments (e.g. shellfish beds) are characterized by phenotypic or genotypic methods and compared to microorganisms known to occur in faeces of nearby human or animal populations in order to make faecal source inferences.

There are a variety of MST methods under active investigation (Scott *et al.* 2002; Simpson *et al.* 2002). Since the field is rapidly evolving, new methods are also being developed (Santo Domingo *et al.* 2007). The collection of MST methods has often been referred to as a toolbox, where some methods may be more relevant than others for a given application. At present, there is no single method that has emerged as clearly superior to all others (Griffith *et al.* 2003; Stewart *et al.* 2003; Stoeckel *et al.* 2004; USEPA 2005), although the selection of a particular method could be influenced by factors like the complexity of the environment under study, the number of sources suspected to be implicated in contamination events, funds available to perform studies, and the technical expertise available to produce and analyze the data.

5.2 IMPORTANCE TO SHELLFISH INDUSTRY

Preventing faecal contamination of shellfish areas is of particular importance because of the close link between water quality sustaining shellfish and the human food supply. The largest viral foodborne outbreak ever reported has been attributed to consumption of clams contaminated with hepatitis A virus from a sewage-polluted area near Shanghai, China, in 1988 (Halliday *et al.* 1991; Tang *et al.* 1991; Potasman *et al.* 2002). Such outbreaks can have significant public health consequences, and significant impacts on local economies. With the development of global food distribution networks for shellfish, it is also possible for local faecal pollution events to have far reaching impacts on shellfish consumers thousands of kilometers away (Berg *et al.* 2000; Sanchez *et al.* 2002).

MST methods are providing an emerging science-based approach for determining the source of faecal pollution contaminating shellfish areas (Meschke and Boyle 2007). Correctly identifying the primary faecal pollution sources could help mediate conflicts among stakeholders in communities, and lead toward appropriate, cost-effective corrective actions to prevent future pollution. It will be important for shellfish managers to track developments relevant to MST to understand the limitations of existing methods and their opportunities for resolving questions about faecal pollution source tracking (Field 2004; US EPA 2005; Stoeckel and Harwood 2007).

5.3 TYPES OF CONTAMINATION

An important aspect of MST is to determine if the source of faecal contamination is of either human or animal origin. The ability to discriminate human and animal faecal contamination is important since it is widely accepted that faecal pollution from a human source (such as sewage) is likely to present a greater human health risk than faecal pollution from animal sources. Many cases of waterborne disease are believed to be of viral origin. Since viruses are often host-specific, many human viruses are likely to occur only in human faecal wastes. However, it is important to also recognize that faecal pollution from animals does not present zero risk (Albarnaz *et al.* 2007). The human health risks from exposure to waters or shellfish contaminated by faecal pollution of animal origin are poorly understood (Till *et al.* 2004). Animal faeces can contain a range of pathogens that can infect humans and waterborne pathogens of zoonotic origin are increasingly recognized as important human health concerns (WHO 2004). Moreover, there may still be pathogens in animal faeces that have yet to be discovered and that could pose significant health risks. Even in cases where the seasonal increase of some pathogens, such as *Vibrio* spp. in coastal waters, is more directly associated with the increase in temperature and nutrient loads during eutrophication events rather than with bacterial flux contributions from human and animal faeces (Epstein 1993; Sack *et al.* 2004), it is still necessary to identify faecal sources as they are in great part responsible for the increases in nutrient loads. Indeed, *V. cholera, V. vulnificus, and V. parahaemoyiticus* are among the most important pathogens in the shellfish industry, impacting many countries worldwide, particularly developing countries.

5.3.1 Human/wastewater

Many areas around the world have inadequate treatment of human faecal wastes prior to their release into water bodies. Untreated human faecal wastes can

contain a wide range of potential bacterial, viral and protozoan pathogens, usually reflecting the health of the community from which they originate. It has been suggested that human enteric viruses are the most common pathogens transmitted by bivalve shellfish (Lees 2000). Formiga-Cruz *et al.* (2002) found human virus contamination of shellfish to be widespread in European waters, and suggested some of these viruses may be good indicators of faecal pollution from human origins. While wastewater treatment processes can reduce pathogen numbers in final effluents, these processes can be of varying effectiveness and they can be prone to system failures or by-passes during high water flows. Most disease outbreaks with oysters have been associated with harvesting from sewage-contaminated water (Potasman *et al.* 2002). In areas where there is inadequate, or perhaps no sewage or septic treatment processes, there can be significant risks of waterborne pathogens contaminating water bodies and shellfish beds. Faecal wastes from ships can also pose threats. Illegal overboard sewage discharges into shellfish harvesting areas have been linked to major outbreaks in the United States (Shieh *et al.* 2003).

One complication when discriminating faecal pollution from municipal wastewater in MST studies is that the wastewater may not contain microbial contaminants exclusively of human origin. Municipal wastewater can contain faecal contamination from food processing activities and domestic animal sources such as pets. In urban areas, stormwater runoff into sewers can contribute faecal pollution from diverse sources such as domestic animals and urban wildlife. For these reasons, caution must be exercised in considering municipal wastewater effluents solely a "human" faecal pollution source.

5.3.2 Domestic animals

Many areas of intensive agricultural production can present risks to shellfish areas from waterborne pathogens originating from livestock faeces. While it is possible to treat livestock wastes and apply manure to agricultural lands to reduce pathogen numbers, poor farming practices or surface water runoff following storms can result in fluxes of faecal pollution and pathogens downstream into estuaries and coastal environments. Gomez-Bautista *et al.* (2000) found *Cryptosporidium parvum* contamination of shellfish only in areas near the mouths of Spanish rivers with a high density of grazing ruminants on their banks. Similar findings were recently reported for clams collected from the mouths of rivers along the Adriatic Sea (Traversa *et al.* 2004). *C. parvum* of bovine origin (i.e. genotype 2) has also been detected in hemolymph and gill washings from oysters in the United States (Fayer *et al.* 1999). In the latter study, aliquots of oysters contaminated with bovine *C. parvum* were found to be infectious in mice.

Clearly, zoonotic agents have important implications to public health and to the shellfish industry. There are currently about 1.2 billion cattle, 800 million pigs and 10 billion chickens around the world, and increasingly intensive rearing practices for domestic animals will present significant animal waste management challenges into the future (Bolin *et al*. 2004).

5.3.3 Wildlife

Faecal contamination from wildlife sources can present an unpredictable and difficult source tracking challenge for shellfish areas. Control of these faecal wastes is not amenable to waste treatment practices similar to sewage treatment or manure lagoons. Where shellfish beds occur near large wildlife populations, consideration needs to be given to monitoring wildlife populations, and their seasonal migrations or behavioural characteristics that could contribute to faecal contamination of shellfish beds. While faecal contamination from wildlife sources is often believed to present low human health risks compared to sewage, wildlife species can carry human pathogens such as *Campylobacter* spp., *Toxoplasma gondii*, and *Giardia* spp. that could pose a health risk. Moreover, zoonotic cryptosporidia genotypes have been detected in samples from infected people (Kassa *et al.* 2004) and waterfowl have been identified as potential carriers of *C. parvum* and *C. hominis*, which are important human protozoan pathogens (Zhou *et al.* 2004). These and other studies demonstrate that the risk of exposure to waters contaminated by faecal pollution from animals such as wildlife is still poorly understood (Till *et al*. 2004; Albarnaz *et al*. 2007).

5.3.4 Other sources

While faecal coliforms and enterococci have long been used as indicators of faecal pollution from humans and warm blooded animals, there is increasing evidence that they can also be associated with non-faecal sources. For example, Bermúdez and Hazen (1988) isolated *E. coli* from water accumulated in the rosette of bromeliads (epiphytes) growing 30 ft above the ground suggesting that the likely source of faecal coliforms in these samples was not mammalian (but may derive from, for example, insects or lizards). Harwood *et al.* (1999) reported *E. coli* in the faeces of cold-blooded turtles. Pulp and paper effluents are known to contain faecal coliforms, including *E. coli* (Gauthier and Archibald 2001). High concentrations of these indicator microorganisms have also been found in tropical soils (Byappanahalli and Fujioka 2004), subtropical sediments (Solo-Gabriele *et al.* 2000), wet sand and

algal mats at temperate freshwater beaches (Whitman and Nevers 2003; Whitman *et al.* 2003), and in drift seaweed along New Zealand marine shores (Anderson *et al.* 1997). In some of these aquatic habitats the indicators may be capable of long term persistence and perhaps reproduction, complicating their use for MST and for indicating recent faecal pollution events (Edge and Hill 2007; Kon *et al.* 2007). Despite many years of study, the ecology of *E. coli* and enterococci still requires additional analysis to better understand the limitations of using these indicators in MST studies (Anderson *et al.* 2005; US EPA 2005).

5.4 METHODS DESCRIPTION

The previous sections have discussed source apportionment; this section discusses the variety of MST methods capable of discriminating among different sources implicated in the faecal pollution of natural water systems (Simpson *et al.* 2002). These MST methods can be classified as library dependent methods (LDMs) or library independent methods (LIMs). In essence, each method or approach has its advantages and disadvantages. The ultimate decision of which method to use relies on several interacting factors, like the availability of resources (funds, trained personnel, equipment), the type of the water system (such as freshwater vs estuarine), the level of contamination (one primary source vs multiple sources), and the level of specificity needed in the study (including human vs nonhuman or domesticated animals vs wildlife). These methods have been used with different levels of success in the laboratory. Results from two independent method comparison studies suggest that some methods can outperform others in predicting faecal sources for samples prepared under laboratory conditions (Stewart *et al.* 2003; Stoeckel *et al.* 2004). However, a comprehensive comparison study has not been performed with environmental samples and therefore it is difficult to exclude any methods as they can all provide useful information under the right set of circumstances.

   The intent of the following sections is to provide a brief description of some of the most commonly used MST methods. Whenever possible, examples of a particular application will be highlighted. In addition, some of the method's limitations will be discussed. A brief summary of some of the MST methods used in the last ten years is provided in Table 5.1. For more detailed descriptions the reader should consult previously published reviews (Scott *et al.* 2002; Simpson *et al.* 2002; Meays *et al.* 2004; USEPA 2005; Field and Samadpour 2007; Harwood 2007; Santo Domingo *et al.* 2007).

Table 5.1 Examples of MST methods

Method	Target tested	Cultivation	Library	References
Antibiotic resistance	*E. coli* *Enterococcus* spp.	Individual isolates	Yes	Wiggins (1996) Harwood *et al.* (2000)
Carbon utilization profiles	*E. coli* *Enterococcus* spp.	Individual isolates	Yes	Hagedorn *et al.* (2003)
rep-PCR	*E. coli*	Individual isolates	Yes	McLellan *et al.* (2003); Dombek *et al.* (2000)
RAPD	*E. coli*	Individual isolates	Yes	Venieri *et al.* (2004)
AFLP	*E. coli*	Individual isolates	Yes	Guan *et al.* (2002)
PFGE	*E. coli* *Enterococcus* spp.	Individual isolates	Yes	Myoda *et al.* (2003)
Ribotyping	*E. coli* *Enterococcus* spp.	Individual isolates	Yes	Myoda *et al.* (2003)
Phage sero-typing and genotyping	F+ coliphage	Individual isolates	No	Cole *et al.* (2003)
Host-specific PCR	*E. coli* toxin genes *E. faecium* esp toxin gene Bacteroides 16S rDNA Bifidobacteria 16S rDNA Metagenomic fragments Enterovirus Adenovirus	Sample enrich-ment or DNA extraction from water sample	No	Khatib *et al.* (2003) Scott *et al.* (2005) Bernhard and Field (2000b) Bonjoch *et al.* (2004) Fong *et al.* (2005) Lu *et al.* (2007)

repPCR, repetitive element polymerase chain reaction; RAPD, random amplification of polymorphic DNA; AFLP, amplified fragment length polymorphism; PFGE, pulsed field gel electrophoresis.
Source: adapted from USEPA, 2005.

5.4.1 Library dependent methods (LDMs)

In LDMs, a library is defined as a database of fingerprints from individual bacterial isolates obtained from potential faecal pollution sources. Bacteria are normally recovered from animal faeces, although faecal samples from animal waste lagoons, septic tanks, and wastewater treatment plants can also be used to create source libraries. LDMs require a cultivation step to obtain the bacterial isolates that will be used to generate the faecal source library (knowns) and the water bacterial isolates that will be challenged against the library (unknowns). To date,

these bacteria have usually been *E. coli* or *Enterococcus* spp. When using *E. coli*, good taxonomical characterization (i.e. to the species level) of the faecal and water isolates is necessary in order to ensure that comparisons used for faecal source identification are appropriate. Most studies using enterococci have relied on multiple species within a group rather than a single species (Wiggins *et al*. 2003).

Bacterial fingerprints are generated using phenotypic or genotypic methods. The most commonly used phenotypic method is based on antibiotic resistance profiles (Wiggins 1996). Other phenotypic methods are based on carbon utilization profiles (Hagedorn *et al.* 2003) and fatty acid methyl esther profiles (Parveen *et al.* 2001). LDM genotypic methods commonly use DNA fingerprinting techniques based on the Polymerase Chain Reaction (PCR) (Dombek *et al.* 2000) or hybridization methods (Myoda *et al.* 2003). While the field is rapidly evolving, most MST field studies in the United States and Canada have been performed using LDMs. This may have resulted from the ease and widespread use of *E. coli* and enterococci in water quality monitoring programs, and the fact that initial laboratory studies reported high average rates of correct classification with known isolates (Dombek *et al.* 2000). Examples of phenotypic and genotypic LDMs are described below.

5.4.1.1 Phenotypic methods

Antibiotic resistance profile

There are three basic approaches to obtaining antibiotic resistance profiles of bacterial isolates for faecal source identification: Multiple Antibiotic Resistance (MAR), Antibiotic Resistance Analysis (ARA) and Kirby-Bauer antibiotic susceptibility. In MAR studies, indicator bacteria have been tested for resistance to a single concentration of different antibiotics (Kaspar *et al.* 1990). In ARA, several concentrations are tested for each antibiotic (Wiggins 1996). The Kirby-Bauer method is based on the disc diffusion assay method which measures the zone of growth inhibition of a given antibiotic on bacterial lawns. In MAR and ARA, multi-pronged replicators can be used to transfer bacterial cell suspensions to the surface of agar plates containing an antibiotic at a specific concentration. The plates are incubated at an appropriate temperature and isolates showing robust growth relative to control plates (same media without antibiotics) are considered to be resistant to the antibiotic in question. The antibiotic resistance approach for source identification is based on the assumption that human and domesticated animals are exposed to different antibiotics and thus gut bacteria will develop resistance to different antibiotics. In contrast, gut bacteria from wildlife should be sensitive to a greater number of antibiotics and at lower concentrations.

ARA has been used in many MST studies since it is relatively inexpensive, enables a high throughput of isolates and does not require much specialized equipment or expertise for a microbiology laboratory (Ebdon *et al.* 2004) (Figure 5.1). It is also one of the few methods that have been applied in subtropical waters (Harwood *et al.* 2000). ARA has also been used to study sources in shellfishing waters. For example, Geary and Davies (2003) argued that several sources were likely to contribute to shellfish contamination along the New South Wales north coast (Australia), although only a very small number of faecal streptococci isolates were examined.

At present, there are no universal standards for antibiotics (or concentrations) recognized for MST studies. The approach to ARA studies may need to vary depending on prevailing medical, veterinary and farming practices used in different countries (including types and doses of antibiotics). Some of the antibiotic resistance profiles that are useful in discriminating among different sources of faecal pollution in one watershed or country, might not be adequate in another one. As a result, antibiotic resistance approaches for MST may not be applicable for resolving complex, multiple host discrimination challenges over larger spatial and temporal boundaries (Moore *et al.* 2005). Like other LDMs, the success of ARA profiling greatly depends on the size and representation of the library used for MST applications (Wiggins *et al.* 2003).

Carbon Utilization Profiles (CUP)

In CUP, bacterial cell suspensions are inoculated into solutions containing different carbon sources and a tetrazolium dye. After cells are incubated, carbon utilization is recorded as presence/absence by monitoring colorimetrically the reduction of the tetrazolium dye. To increase the throughput, reactions can be performed in 96-well plates generated in house or by using commercially available microplates (Biolog, California, USA; PhPlate, Stockholm, Sweden). Although the use of CUP in source identification has been limited to a couple of studies (Hagedorn *et al.* 2003), like ARA, it should be noted that the type of resources/equipment needed to generate isolates profiles are normally part of conventional microbiological laboratories. Visualization of CUP can be automated with relatively inexpensive image readers.

Fatty Acid Methyl Esther Profiles (FAME)

FAME analysis is based on the analysis of membrane fatty acids. This method has been used regularly to identify bacterial groups in various environments and to compare the overall structure of naturally occurring microbial communities (Glucksman *et al.* 2000; Banowetz *et al.* 2006; Quezada *et al.* 2007). As an MST
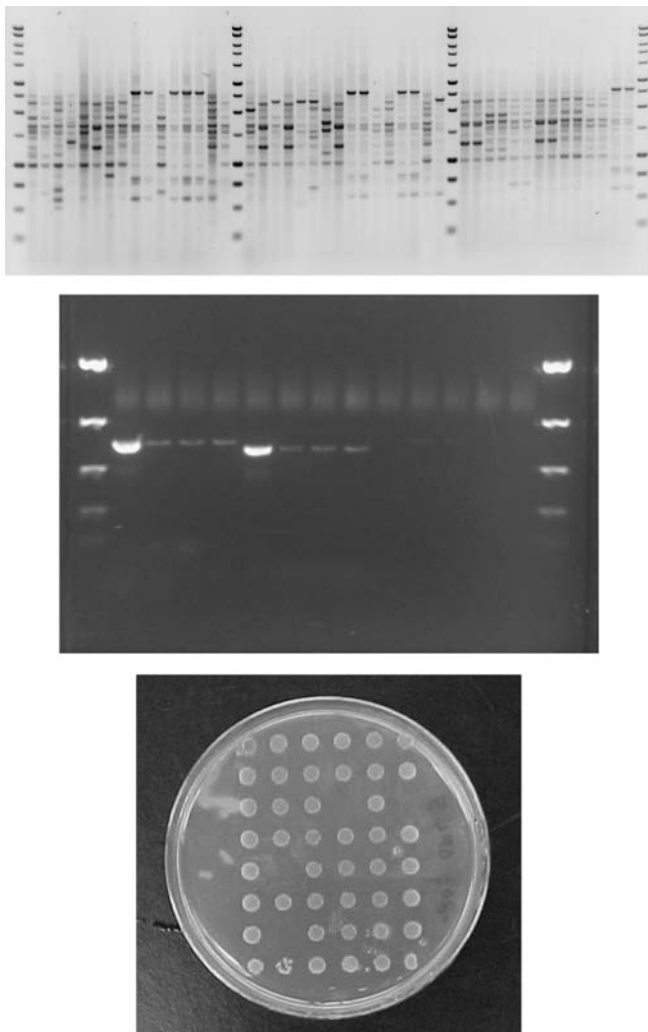
Figure 5.1 Different methods commonly used to identify sources of faecal contamination: ARA (top), rep-PCR (middle) and 16S rDNA Bacteroides host-specific assay (bottom). ARA is an example of a LDM phenotypic method. In this case, most isolates are resistant to the particular antibiotic in the media. rep-PCR is an example of LDM genotypic method. Several bands are associated with each pattern. Theoretically, *E. coli* isolates with similar banding patterns may come from the same faecal source. The *Bacteroides* spp. host-specific assay is an example of a LIM that does not require a pre-enrichment step and that produces a host-specific DNA fragment. (ARA photo was provided by Bruce Wiggins.)

method, the fatty acid fraction is extracted from faecal coliforms or *E. coli* isolates grown on solid media plates for 24 hours and the profile of fatty acids is then determined using gas chromatography analysis. Only a few laboratories have used FAME analysis for faecal source identification. For example, Duran *et al.* (2006) suggested that FAME profiles could be used to discriminate between faecal coliforms of human and non-human origin. In contrast, Parveen *et al.* (2001) showed that there was no relationship between profile and isolate source when discriminating human sources and nonhuman sources of *E. coli*. The latter results indicate that isolates from the same bacterial species are likely to share many of their membrane fatty acids, particularly after they are grown on the same artificial medium. While the lack of host-specific signature fatty acids in different faecal *E. coli* isolates was also recently documented by Haznedaroglu *et al.* (2007), the authors argued that relative masses of certain FAMEs still have source tracking value.

5.4.1.2 Genotypic methods

rep-PCR

Repetitive element sequences have been used in PCR assays since the early 1990's to perform genotypic characterization of Gram-negative and Gram-positive bacteria (Versalovic *et al.* 1991). These repetitive sequences have been used to ascertain the diversity of clinical and environmental bacterial isolates. Several types of repetitive elements have been identified in bacteria, three of which have been used in faecal source identification studies: repetitive extragenic palindromic (REP) sequences, enterobacterial repetitive intergenic consensus (ERIC) sequences, and BOX sequences (Scott *et al.* 2002). Collectively, these methods are known as rep-PCR. *E. coli* has been the primary organism used in rep-PCR MST studies. The general steps for rep-PCR are isolation of bacterial DNA, amplification using REP, ERIC or BOX primers, separation of different sized DNA fragments by gel electrophoresis, image capturing and DNA fingerprint analysis (for example using Bionumerics software). Many MST-related studies using rep-PCR are found in the scientific literature (Dombek *et al.* 2000; McLellan *et al.* 2003). One of the advantages of this method is that it is a relatively simple molecular method which does not require expensive equipment other than a PCR thermocycler. However, problems associated with poor technical reproducibility among different laboratories has been reported to occur (Johnson and Clabots 2000) which is problematic for comparison purposes and in the development of standardized methods. Recent improvements in rep-PCR have included changes in the reaction chemistry and thermal cycling conditions, incorporation of microfluidics

for PCR product fractionation and detection, and computer-assisted analysis (Johnson *et al.* 2004; Healy *et al.* 2005).

Ribotyping

Ribotyping is based on the analysis of restriction fragment length polymorphism (RFLP) fingerprints. These patterns are generated by digesting genomic DNA of bacterial isolates followed by a Southern hybridization step using the rRNA gene operon as the hybridization probe. Once the hybridization is performed, DNA band patterns are captured using digital cameras. Image analysis to compare DNA fingerprints is performed using commercially available software (such as Bionumerics). Like rep-PCR, *E. coli* has been the most common organism in MST studies using ribotyping. To increase the level of accuracy of this technique in source tracking applications it may be necessary to use more than one restriction enzyme (Scott *et al.* 2003). In a recent comparison study, ribotyping was one of the best performing techniques in terms of correctly classifying unknowns, although it was unable to classify many isolates (Stoeckel *et al.* 2004). Compared to PCR-based techniques, ribotyping is more expensive and technically demanding.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE requires a special agarose gel rig with multiple electrical fields to separate large DNA fragments digested *in situ* with restriction enzymes. Enzymatic digestion of DNA is performed in agarose plugs containing whole cells. Plugs are transferred to agarose gels and DNA fragment separation is then achieved by applying short electrical pulses from switching electrical fields. All the other steps are very similar to other gel electrophoresis methods used in MST, like rep-PCR (Myoda *et al.* 2003). PFGE is considered the gold standard method in epidemiological research. In theory, the discriminatory power of PFGE could be of great value in faecal source identification. However, PFGE has not been widely used in MST due to the fact that it is not amenable to rapidly generating large library databases. Moreover, due to its strong discriminatory power the number of fingerprints that need to be analyzed to match water and faecal isolates is often larger than other molecular methods (such as rep-PCR and ribotyping).

Density Gradient Gel Electrophoresis (DGGE)

DGGE is a gel electrophoresis technique that separates PCR products of similar length but different nucleotide composition. Polyacrilamide gels containing a urea gradient are used instead of the conventional agarose gels and primers containing GC-clamps are used in the PCR assays to facilitate the separation of

PCR populations. DGGE has been used with primers targeting the 16S-23S rRNA intergenic spacer region of *E. coli* strains isolated from two streams and from bovine, poultry and human sources (Buchan *et al.* 2001). Due to the high diversity of isolates with different DGGE profiles the authors concluded that it would be difficult to use this approach to unambiguously identify faecal pollution sources. This is in contrast with a more recent study that suggests that host-specific rDNA-based DGGE patterns can be obtained with a relatively small number of strains isolated per source (Seurinck *et al.* 2003). Recent MST DGGE-based approaches have also targeted the beta-glucuronidase gene (uidA) of *E. coli* (Lasalde *et al.* 2005; Sigler and Pasutti 2006).

Amplified Fragment Length Polymorphism (AFLP)

AFLP is based on the arbitrary amplification of restriction fragments ligated to double-stranded adaptors. This technique has been used in many bacterial typing applications, although it has only recently been used to discriminate among strains isolated from different groups of animals in laboratory studies. Guan *et al.* (2002) used AFLP to classify livestock, wildlife isolates and human isolates. As one of the primers used in the PCR step can be fluorescently labeled, AFLP is therefore amenable to automation, which greatly increases its throughput capability.

Random Amplification of Polymorphic DNA (RAPD)

This technique is similar to rep-PCR as randomly distributed genomic fragments are amplified via PCR, which are then analyzed using agarose gel electrophoresis and image analysis. However, fingerprints are generated using primers (generally 10 base pairs in length) carrying arbitrary sequences. Venieri *et al.* (2004) reported three RAPD-based assays that produced distinct profiles between *E. coli* isolates from humans and animals. However, this method has been reported to be very sensitive to buffer conditions, which could affect reproducibility and technology transfer.

Gene sequencing

Sequencing analysis of 16S rRNA, beta-glucuronidase, and malate dehydrogenase genes has been used to look for host-specific signature sequences (Ram *et al.* 2004; Simpson *et al.* 2004; Ivanetich *et al.* 2006). Although it is becoming more routine in research laboratories, gene sequencing is time consuming, technically demanding and the analysis of the data is somewhat laborious. However, if useful sequences are obtained it is possible to use this information in the development of host-specific PCR-based methods (Dick *et al.* 2005). Sequencing information has also been useful at confirming the presence

of environmental clones exhibiting different levels of host-specificity (Lamendella *et al*. 2007).

5.4.1.3 Limitations and critical issues regarding LDMs

LDMs based on faecal indicator bacteria can have advantages when communicating results of MST studies to shellfish managers who make water quality decisions using these same indicator bacteria. However, limitations of LDMs need to be recognized. Some of the limitations associated with LDMs are the need for cultivation and species identification, large library sizes, and the complexity of the statistical analyses. Many of these methods have been used successfully in taxonomical and epidemiological studies but have not been widely applied in MST-field studies. While LDMs are attractive because they can provide quantitative information on the relative proportion of inputs from different faecal sources, the accuracy of these quantitative predictions still needs to be tested. Most LDMs can be useful on small study scales. However, the geographic and temporal stability of fingerprint libraries remains unknown.

One of the most critical aspects of LDM-based studies is the library size. It is important to note that many of LDM studies have been performed with relatively small libraries. While there is no consensus regarding the minimum number of faecal isolates needed for reliable source identification, the number could be quite large. For example, Jenkins *et al*. (2003) suggested that a library of anywhere from 900 to 2000 faecal isolates would be needed in order to represent the number of transient and resident *E. coli* ribotypes of two Black Angus herds (beef cattle). Hence, in cases in which there are multiple faecal sources it is necessary to consider the development of libraries containing hundreds (and perhaps thousands) of isolates per source to obtain a good representation of the diversity of faecal clones present in a study area. In general terms, the fewer faecal source isolates used in an MST application the less likely the method is to reliably classify unknowns (as in water isolates). Additionally, disproportional representation of clonal genotypes and isolates from a particular source(s) in a library can have an impact on the statistical outcome of LDM-based MST studies (Johnson *et al*. 2004; Robinson *et al*. 2007).

The number of water isolates is also important in LDM studies and could be prohibitively large depending on the sampling scheme and the complexity of the water system. The latter is particularly true in some cases due to the presence of non-faecal isolates (i.e. soil and plant isolates) which can often introduce an undetermined number of unknowns which cannot be correctly classified regardless of the size of the library. In fact, the persistence of indicator bacteria in secondary habitats (i.e. outside of the gut environment) has recently

been the object of several investigations (Gordon *et al.* 2002; Whitman and Nevers, 2003). Some of these issues could be addressed by understanding the background microbiota in potential non-faecal sources.

5.4.2 Library independent methods

Due to factors like the difficulty of building a sufficiently robust library and the complexity of data analysis, the focus of much recent MST research has shifted towards the development of library independent methods (LIMs). Normally, LIMs do not require a cultivation step, although in some cases a cultivation step is necessary to increase the densities of the microbial species carrying the targeted gene. As a result, LIMs could be generally classified as culture dependent or culture independent. Among the bacterial genes used as source identification markers are antibiotic resistance genes (e.g. *tetA*), phylogenetic genes (e.g.16S rDNA), and virulence genes (e.g. LTIIa). Viral genes have also been used in faecal source identification (Pina *et al.* 1998; Fong *et al.* 2005). Most LIMs rely on PCR to detect host-specific markers (Figure 5.2), although DNA hybridization has been used with viral genes (Hsu *et al.* 1995).
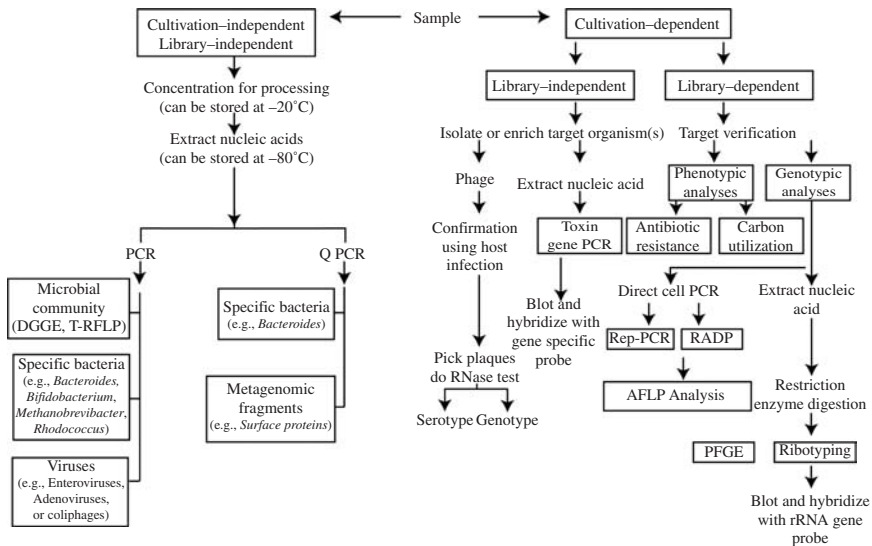
Figure 5.2 Steps involved in some of the different types of MST methods. Source: adapted from USEPA 2005.

In theory, a LIM-based approach is relatively simpler than a LDM-based approach since the development of a large library is not required. This allows MST practitioners to increase the number of water and faecal samples that could be processed in the laboratory, which in turn increases the statistical relevance of the results. However, a significant amount of validation is necessary to ascertain the relative abundance and distribution of the marker (in the targeted host) and the potential presence of false positive reactions (in non-targeted hosts). Validation studies should be performed by challenging the assay(s) against nucleic acid extracts of individual faecal samples from different hosts. Some of the recently developed assays are described below.

5.4.2.1 Culture dependent LIMs

LTIIa and STII PCR assay

The toxin genes LTIIa and STII from enterotoxigenic *E. coli* (ETEC) have been used in PCR assays to identify the presence of cattle and swine faecal pollution (Khatib *et al.* 2002; Khatib *et al.* 2003). LTIIa and STII PCR assays were found to be highly host-specific after these were challenged against 221 and 110 DNA extracts from different animal faecal and human sewage samples, respectively. One caveat of this approach is the need for relatively high numbers of ETEC strains to occur in the water samples for the assays to be effective. Since ETEC strains are normally found in low densities in environmental waters, a cultivation step is required to increase the sensitivity of these assays.

esp PCR assay

Scott *et al.* (2005) developed a human-specific PCR assay that targets the *esp* gene, a virulence factor in *Ent. faecium*. This method is similar to the LTIIa and STII PCR assays in the sense that water samples are filtered onto membranes which are placed onto selective media agar plates and incubated for a defined period of time (normally overnight). Membranes are then transferred to liquid medium and incubated for a short period of time to increase bacterial biomass. Cells are washed off the membranes and DNA extracts of the washed cells are used in PCR assays. Using this approach Scott *et al.* (2005) showed that 97% of sewage and septic samples produced a positive signal while no PCR products were detected in any livestock waste lagoon samples or in bird or animal faecal samples. However, results from a recent study showed a lower incidence of the *esp* gene marker in human sources and assay cross reactivity with some animal faeces (including dogs, gulls, mice and songbirds), questioning the discriminatory power of this specific assay (Whitman *et al.* 2007).

Bacteriophages

Most MST-related studies using bacteriophages have focused on viruses that infect *E. coli* (Hsu *et al.* 1995; Cole *et al.* 2003) and *Bacteroides* spp. (Blanch *et al.* 2004; Ebdon *et al.* 2007). Methods targeting male-specific bacteriophages (MSB) of *E. coli* have been developed to discriminate between human and non-human sources, while assays targeting *Bacteroides* spp. phages have been used as human-specific assays. Phage typing of *Salmonella enterica* isolates was recently used to determine contamination sources in two commercial pig slaughter-houses (Botteldoorn *et al.* 2004). Bacteriophages have been suggested to be better surrogates of viral pathogens than conventional indicator bacteria (Havelaar *et al.* 1993). However, poor coexistence between bacteriophages and enteric viruses was documented for mussels collected from coastal sites in Sweden (Hernroth *et al.* 2002). Additionally, MSB densities in animal faeces are relatively lower than in humans (Calci *et al.* 1998), reducing the sensitivity of phage-based methods in MST field applications, particularly in waters impacted with animal faecal sources.

5.4.2.2 Culture independent LIMs

16S rDNA assays

Recent efforts have focused on the use of 16S rDNA (16S rRNA gene) of faecal bacteria as the target for host-specific markers (Matsuki *et al.* 2004a; Simpson *et al.* 2004). The 16S rDNA is present in all bacteria and is routinely used in bacterial phylogenetic studies. Some of the most promising results for 16S rDNA marker development have been obtained with the *Bacteroidetes* family. For example, using terminal-RFLP (t-RFLP) and primers specific to *Bacteroides*-like bacteria, Bernhard and Field (2000a) showed the presence of bacterial host-specific 16S rDNA sequences in human and cow faecal samples. In a follow-up study, Bernhard and Field (2000b) applied *Bacteroides* 16S rDNA PCR assays specific to ruminants and humans. More recently, Simpson *et al.* (2004) showed the presence of a horse-specific 16S rDNA *Bacteroides*-like cluster, further supporting the potential use of this bacterial group to track sources of faecal pollution. The continuous increase in publicly available 16S rDNA sequences will provide additional signature targets for host-specific assays (Dick *et al.* 2005).

Bonjoch *et al.* (2004) recently developed a multiplex-PCR assay specific to human sewage. In their study they evaluated the presence of nine human-related *Bifidobacterium* species using 16S rDNA PCR assays. The assays were challenged against cattle, swine, poultry and human faecal sources. The results showed that only *B. adolescentis* and *B. dentium* were exclusively found in human sewage samples. Other bifidobacteria 16S rDNA-based PCR assays have been developed (Mullié *et al.* 2003; Matsuki *et al.* 2004b), although most of

these assays have not been challenged against animal faeces, so it is not known if they are valuable in MST studies. However, Lamendella *et al.* (2008) recently showed that bifidobacterial species previously suggested as indicators of human faecal pollution (*Bifidobacterium adolescentis, Bif. bifidum, Bif. dentium*, and *Bif. catenulatum*) can be broadly distributed in different animals. In the same study, no bifidobacterial signals were detected in waters with history of faecal pollution and that were positive to other alternate faecal indicators (i.e. *Bacteroidetes* and *Clostridium coccoides*). Hence, bifidobacterial assays might have limited value in MST field applications.

A total faecal community t-RFLP approach was recently compared to other culture-independent LIMs (Field *et al.* 2003). This method uses universal primers to amplify 16S rDNA directly from total faecal DNA, and as a consequence, due to the predominantly anoxic conditions of the gut, signature signals are likely to belong to strictly anaerobic bacteria like *Bacteroides* spp*., Clostridium* spp., *Eubacterium* spp., *Bifidobacterium* spp*., Fusobacterium* spp., *Butyrivibrio* spp*., Pseudobutyrivibrio* spp. and *Ruminococcus* spp., among others. While the method was capable of discriminating among different faecal microbial communities, it is possible that the host-specific sequences will be difficult to detect within the background of non-faecal bacteria also inhabiting faecally contaminated waters.

Non 16S rDNA assays

While most culture-independent LIMs have used *Bacteroidetes* 16S rDNA sequences, alternate genetic targets have recently been tested. For example, the dinitrogenase reductase gene (nifH) of the archaea *Methanobrevibacter ruminantium* and *M. smithii* have been used to develop ruminant- and human-specific assays respectively (Ufnar *et al*. 2006; Ufnar *et al*. 2007a). Other archaeal genes (mcrA) as well as faecal metagenomic fragments presumed to be encoding for functional genes have also been used in assay development (Lu *et al*. 2007; Ufnar *et al*. 2007b). Assays targeting functional genes have shown high levels of host specificity. However, the detection limits of the latter assays are relatively lower than assays targeting multiple copy genes (like rRNA genes) in environmental waters, which might narrow their use to specific field applications.

Enteric virus assays

Several methods are available for the detection of bacteriophages and eukaryotic viruses. More importantly, monitoring human viruses has been suggested as an alternate approach to assess human health risks in recreational waters (Jiang *et al*. 2001) and for faecal source identification studies (Girones 2006). For example, Maluquer de Motes *et al.* (2004) developed bovine and

porcine adenoviruses assays to determine the occurrence of animal viruses in sewage samples. The results showed the presence of porcine and bovine adenoviruses in at least 70% of the pooled animal samples tested, but no signals were detected in urban sewage samples further confirming the host specificity of the assays. In another study, Fong *et al.* (2005) detected human enteroviruses, human adenoviruses and bovine enteroviruses in approximately 37, 57, and 37% respectively of surface water samples collected near shellfish harvesting areas. The presence of these viruses did not correlate significantly with faecal coliforms or total coliform levels, suggesting that the latter bacterial indicators could underestimate risks associated with enteric viruses in these waters.

Tetr PCR assays

PCR-amplification of antibiotic resistance genes has been used to determine the presence of tetracycline resistance (tet^r) genes in feedlot lagoons and animal faeces (Chee-Sanford *et al.* 2001; Smith *et al.* 2004). The results from these studies suggest that this approach could be used to detect the impact of livestock faecal pollution on nearby water bodies. However, the presence of tet^r genes in non-faecal environmental bacteria suggests that these genes can be mobilized from faecal bacteria to groundwater bacteria. The background of native tet^r bacteria (Koike *et al.* 2007) could clearly represent a potential problem for this source tracking approach.

5.4.2.3 Limitations and critical issues regarding LIMs

One of the current limitations of LIM approaches is the lack of methods for host species beyond humans and a few important domestic animal species. While a number of LIMs have been reported in the literature, their use in field applications has been limited. In most cases, the validation of these markers has been performed against only a few different hosts and a limited number of individual faecal samples. Moreover, the ecological significance of most of the targeted genes is not well understood. Therefore, the distribution and host-specific nature of most LIM-based markers is yet to be determined. In addition, the relative abundance of most of these molecular markers has not been assessed. The latter information is needed in order to better estimate faecal loads and better understand the presence of false negative signals. To date, LIMs have provided a presence-absence characterization of host-specific contributions to faecal pollution. However, many shellfish managers would like quantitative information on the relative proportion of inputs from different faecal sources. In shellfish areas with multiple faecal pollution sources, this will remain a limitation of LIMs until quantitative methods are better developed.

5.4.3 Targeted organisms for faecal source identification

5.4.3.1 Bacteria

Conventional indicator bacteria

E. coli and *Enterococcus* spp. have been the target of choice in the vast majority of MST studies to this date. Swimming advisories are posted and waters are banned for shellfishing based on the levels of these indicators of faecal pollution. Consequently, identifying the sources of these bacteria has been the focus of most studies thus far, primarily because they are the basis of microbial water quality criteria. These bacteria, considered common inhabitants of most warm-blooded animal guts, are relatively easy to culture. Selective media are available for their isolation, which minimizes the number of false positives that need to be further characterized. Most studies using these bacterial indicators have relied on LDM-based approaches. However, virulence genes of *E. coli* and *Ent. faecium* have been used in a library-independent fashion to detect animal and human contamination (Khatib *et al*. 2003; Scott *et al*. 2005). In addition, a few studies have suggested that the presence of *Ent. faecalis* alone can be used as an indicator of particular sources of contamination (Kuntz *et al.* 2003; Creti *et al.* 2004).

Faecal anaerobic bacteria

Bacteroides spp. and *Bifidobacterium* spp. are obligate anaerobic bacteria found in the gastrointestinal tract of humans and warm-blooded animals. In contrast with other faecal bacteria, members of these genera are believed to exhibit low survival rates in water. Until recently these bacterial groups were commonly detected using strictly anaerobic cultivation techniques. Since their detection is now possible with PCR they are now considered as potentially practical indicators of recent faecal contamination. Several *Bacteroides* species are believed to be normal members of the human gut, particularly *B. thetataomicron*, *B. ovatus*, and *B. vulgatus*. Similarly, the bifidobacterial species *Bif. adolescentis*, *Bif. angulatum*, *Bif. bifidum*, *Bif. breve*, *Bif. catenulatum*, *Bif. infantis*, *Bif. longum*, and *Bif. pseudocatenulatum* have been routinely isolated from child and adult faeces suggesting that these are good sources of human markers (Matsuki *et al.* 2004a). As previously mentioned, 16S rDNA assays targeting both genera have been suggested as potential faecal source identification tools. For example, Nebra *et al.* (2003) recently developed two bifidobacterial-based assays: one human-specific assay that targets *B. dentium* and another that is specific to cattle and goats. Thus far, the latter method has only been performed in a limited number of laboratories and additional studies are needed in order to further assess its potential against complex water systems. One potential concern with the use

of *Bacteroides* spp. and *Bifidobacterium* spp. is their allegedly poor survival rates outside of the animal gut. However, this is a useful characteristic when trying to discriminate between recent and less-recent contamination events.

Rhodococcus coprophilus has been suggested as an indicator of nonhuman faecal contamination due to its frequent isolation in animal faeces and common absence in human faeces (Mara and Oragui 1981). PCR and real-time PCR methods for *R. coprophilus* (Savill *et al.* 2001) have been developed. Due to their preferential distribution in herbivores, assays specific to *R. coprophilus* could be used as part of an MST toolbox. However, the high survival rates can diminish their value in cases relevant to recent faecal contamination events (Oragui and Mara 1983).

The ecology of conventional indicator bacteria like *E. coli* has been studied in some detail. For many other organisms the data is somewhat cursory and outdated. However, even though the ecology of conventional indicator has been studied for decades, new knowledge continues to surface about possible limitations of their use for faecal source identification purposes. In addition, more comprehensive sequence databases are needed for the genes targeted in LIMs, as successful marker detection depends on perfect sequence matching. This is particularly relevant to real-time PCR methods aimed at the quantification of targeted DNA sequences (Seurinck *et al.* 2005).

5.4.3.2 Viruses

Phages

Bacteriophages or phages are bacterial parasites that are known to play a role in the population dynamics, genetics, and evolution of their host. Due to their importance in genetics, coliphages (phages that infect *E. coli*) represent the most studied bacteriophage group. This group has also been suggested as an alternate indicator of faecal contamination due to its similar survival rate to enteric viruses (Havelaar *et al.* 1993). A couple of recent studies have suggested the potential use of four F+ RNA coliphages as targets in MST studies. The presence and/or proportions of these four coliphage types have been noted to be different depending on the type of faecal contamination. For example, types II and III are often associated with human contamination while the occurrence of types I and IV is more prevalent in animal faeces. However, a recent study showed that group III is also common in swine faeces (Cole *et al.* 2003). The fact that these coliphage groups are present in more than one animal type suggests that they are not completely host-specific and therefore that assays based on these phage groups might not conclusively be able to discriminate between faecal pollution of human and animal origin.

Phages infecting *B. tethaiotaomicron* and *B. fragilis* have also been suggested for MST-applications, specifically for detecting human faecal sources (Muniain-Mujika *et al.* 2003; Blanch *et al.* 2004). This is a relatively recent approach that has not been validated in many laboratories. *Bacteroides* species are anaerobic bacteria, special techniques and equipment are needed in order to detect this phage group. This might preclude the routine use of this approach in many laboratories.

Eukaryotic viruses

Eukaryotic viruses are perhaps one of the most logical targets for the development of novel host-specific markers due to the narrow host range of this microbial group. In addition to their use as targets for MST markers, viral markers can also be used to assess public health risks. Not surprisingly, human enteric viruses are present at high densities in human waste and therefore they might be useful indicators of wastewater treatment plant effluents in receiving waters. Two classes of eukaryotic viruses have been primarily used in MST related studies: the enteroviruses and adenoviruses. Due to the low titers in natural waters, pre-concentration of up to 100 L of water may be required in order to detect a viral signal. This has limited their use and evaluation in field studies. However, Fong *et al.* (2005) recently detected human enteroviruses, human adenoviruses and bovine enteroviruses using reverse transcription- and nested-PCR assays in relatively small sample volumes (2 L).

Although the host specificity associated with many viruses makes them attractive faecal pollution source tracking tools, there are challenges to using viral markers for faecal source tracking. These markers may not be prevalent in all members of a given host population and viral particles may occur only in very low numbers in natural waters. The temporal and geographic stability of these viral markers remains to be well tested. For example, viral methods may be capable of identifying water samples contaminated with sewage but may not be capable of identifying water samples contaminated with septic tanks (Griffith *et al.* 2003).

It is important to note that more information is needed regarding the ecology and the host range of animal and human enteric viruses to better assess the value of emerging viral methods within the source identification toolbox. This information is critical as some studies are suggesting a wider host range for enteric viruses than previously known. For example, Varghese *et al.* (2004) found examples of rotavirus genes of porcine origin associated with infantile gastroenteritis in Manipur, India. Moreover, two of the genes tested are thought to be involved in host range restriction and pathogenicity. Other studies have shown that rotaviruses of bovine origin are associated with asymptomatic

infections in children (Varshney *et al.* 2002). While the rates of cross-transmission of enteric viruses are believed to be extremely low in developed countries, in regions exhibiting lower standards of sanitation the incidence of transmission is expected to be higher. However, it should be noted that enteric viruses of animal origin have been detected in drinking water of developed countries via reverse transcription-PCR analysis (Gratacap-Cavallier *et al.* 2000).

5.4.3.3 Protozoa

Cryptosporidium spp. are protozoa that occur as parasites of animal gut cells. These protozoa are also pathogens responsible for waterborne disease outbreaks. In a series of studies, Xhiao and colleagues demonstrated host specificity of *C. parvum* strains based on 18S rDNA sequences (Xiao *et al.* 2000). Moreover, using *Cryptosporidium*-specific 18S rDNA-based assays, the same research group showed that surface waters in the USA are predominantly contaminated with *Cryptosporidium* spp. from human, cattle, and wildlife faecal origin (Xiao *et al.* 2001). Other studies have combined *Cryptosporidium* spp. source tracking with land use analyses to determine prominent sources of faecal pollution (Ruecker *et al.* 2007). Like their viral counterparts, *Cryptosporidium* spp. are not abundant in natural waters and therefore their detection relies on concentrating large volumes of waters (i.e. 20–100 L). This has precluded their widespread use in source identification studies.

5.4.3.4 Alternate targets

Eukaryotic markers

The limited availability of bacterial host-specific markers has encouraged several laboratories to look directly at host cells as a source for host markers. A PCR method based on mitochondrial gene sequences was developed by Martellini *et al.* (2005) to differentiate human, bovine, porcine and ovine sources in faecally contaminated surface water. The rationale for using this approach is based on the significant differences in mitochondrial sequences between different animals. As host cells are sloughed off into the gastrointestinal tract these are then excreted with faeces and therefore reach environmental waters faecally impacted by any particular animal type. A similar approach has been used in the food industry to detect specific animal species in food. One possible problem is the potentially poor survival of eukayotic cells in environmental water systems. However, Martellini *et al.* (2005) detected human, cattle, and swine signals in run-off water samples with these assays, suggesting that this approach might be useful to detect recent faecal pollution events.

Chemical markers

While microbial targets have been the focus of much recent method development, chemical targets have also been used to trace faecal contamination in natural waters (Edwards *et al.* 1998) and sediments (Isobe *et al.* 2002). Most chemical approaches have focused on the detection of chemicals that are associated with anthropogenic activities, although similar approaches have been proposed in the development of guidelines for feedlot manure and effluent management practices (Khan *et al.* 2007). As the highest concentration of many of these chemicals is found in wastewater treatment plants, they have been proposed as adequate markers of human faecal waste pollution. Caffeine, detergents and fragrance materials are some of the chemicals suggested as good human markers. In addition, the use of fluorometry to detect laundry brighteners in surface waters was recently tested during baseflow and stormflow conditions (Hartel *et al.* 2008).

Faecal sterols and faecal stanols are promising markers of faecal pollution (Chan *et al.* 1998; Elhmmali *et al.* 2002). Some of these compounds show host specificity, thus their detection could be used to suggest the presence of particular pollution sources. More recently, the ratio of different types of sterols and stanols has been used as a chemical index of host-specific faecal sources (Chou and Liu *et al.* 2004). The basic premise is based on the presence of unique stanol and sterol fingerprints for each different animal type. Even though some of these compounds might be present in different hosts, their ratio varies significantly. Each host-specific ratio could then be used to suggest primary faecal pollution sources (Roser *et al.* 2003).

Another chemical compound of potential source tracking value is the faecal secretory immunoglobulin A (sIgA). Faecal sIgA is present in the intestinal mucosa of animals and as a result present in animal excreta. Theoretically, the levels of sIgA should vary depending on the level of pollution, and therefore it may be a good target for source identification in faecally impacted waters. In cases where the contamination is relatively low, concentration of large volumes of water is required. The detection of sIgA is performed using enzyme-linked immunosorbent assays. While many laboratories have reported methods to extract sIgA from faecal samples (Franz and Corthier 1981; Peters *et al.* 2004; Michalsen *et al.* 2005), thus far no studies have been published on the detection of sIgA from environmental waters.

There are some significant issues to be considered with the use of chemicals when tracking sources of faecal pollution. For example, the fate of these organic chemicals in environmental waters has not been studied in great detail. Both degradation and adsorption processes could result in their fate being quite different

from microorganisms in sources of faecal pollution. Secondly, the large volumes of water required for the downstream analysis significantly limits the number of samples that can be analyzed in any given study. Consequently, sources may need to be determined with much fewer samples than some of other MST methods. Thirdly, additional information regarding the host distribution and host specificity of many of these chemicals is also needed in order to understand their value in source identification in complex water environments.

5.4.4 Targeted sampling

A relevant impediment in achieving source identification relates to the significant amount of resources that are needed in order to correctly pinpoint the most probable sources of faecal pollution. In many cases, the equipment needed to perform DNA fingerprinting is relatively expensive and found only in laboratories that conduct molecular biology studies. Recently, Hartel and colleagues (Hartel *et al.* 2008) have suggested that the use of targeted sampling could significantly reduce operational costs while identifying primary sources of faecal pollution in surface waters (McDonald *et al.* 2006). Targeted sampling relies on spatial and temporal analysis of bacterial indicator levels to identify the "hot spots" or areas of highest pollution in conjunction with detailed sanitary survey inspection of heavily impacted sites. Once a reduced number of hot spots are identified, any of the MST methods could then be used locally to confirm the source of the contamination. Using this approach, Kuntz *et al.* (2003) were able to show, relatively quickly and cheaply, that a broken pipe was responsible for faecal contamination. A recent approach using fluorescence detectors was coupled with targeted sampling to detect human sources of contamination in subtropical waters (Hartel *et al.* 2008).

5.5 DECIDING WHICH METHOD TO USE

At present, it is not possible to recommend a standard MST approach and method that would be applicable to all faecal pollution source tracking situations pertaining to shellfish areas. The decision of which MST method the shellfish manager should use to identify sources of faecal contamination could vary among shellfish areas. Even for relatively simple faecal contamination problems, the decision will be influenced by a number of factors, ranging from available funds to availability of personnel with technical knowledge. Time is another important factor as some methods can provide relatively accurate answers but require considerable time for sample processing and data analysis. The

following questions were used as decision criteria in a recent MST guide (US EPA 2005) for guiding selection of appropriate MST approaches.

- Is the problem adequately defined?
- Are the desired outcomes identified?
- What are the typical hydrological conditions in the watershed?
- Has an adequate sanitary survey been conducted?
- How many faecal sources were identified in the sanitary survey?
- Is the watershed/study area of manageable size?
- What is the desired level of faecal source discrimination?
- What type of resources are locally available (e.g. laboratory capabilities)?

Shellfish managers and scientists should answer most of these questions in order to effectively decide whether and how to apply MST methods. Many of the issues pertaining to the selection of an appropriate method are addressed in greater detail elsewhere (US EPA 2005).

5.6 FAECAL SOURCE IDENTIFICATION IN DEVELOPED AND DEVELOPING COUNTRIES

Distinguishing between human and non-human faecal contamination is gaining importance worldwide in light of the impact of faecal pollution on human health and economic affairs. In many developed countries, regulatory agencies are establishing programs to deal with non-point polluting sources which are primarily responsible for impacting surface waters. However, in developing countries, source identification has not been as driven by regulatory actions, in spite of the fact that faecal pollution of surface waters continues to be a major problem in many countries in South America, Asia and Africa.

The links between faecal contamination and waterborne and foodborne outbreaks are well established. For example, the quality of surface water is relevant to public health due its use as a source of drinking water, recreational activities and food production. Accurate assessment of the primary sources of faecal pollution is clearly needed in order to implement and evaluate management practices to effectively remediate faecally polluted waters and reduce food contamination. Moreover, accurate source identification methods are needed to calculate the different risks associated with each of the sources impacting any given water system. While it is possible that the health risks associated with different faecal sources might vary between developing and developed countries, a significant increase in the use of source tracking

techniques in developing countries is needed in order to understand any potential differences.

Many MST methods have been applied only to faecal pollution problems in temperate freshwater ecosystems of developed countries. It is uncertain whether the experience gained from this research can be readily extrapolated to other areas such as shellfish beds in tropical marine ecosystems of developing countries. In theory, many of the MST tools could be adapted to all types of geographical locations. However, the reported natural occurrence and prolonged survival of enteric bacteria in tropical environments could have an impact on the effectiveness of some of the source identification methods that rely on indicator bacteria (Santiago-Mercado and Hazen 1987; Santo Domingo *et al.* 1989; Byappanahalli and Fujioka 2004). For this reason, alternate indicators with lower survival rates than conventional indicator bacteria might be better suited for source identification in tropical countries.

In addition, there is still a lot to learn about the ecology of indicator organisms used as faecal source identifiers. Despite many decades of water quality research in temperate freshwater environments, some limitations of using *E. coli* for source tracking are still coming to light. Worldwide microbial water quality is normally performed using conventional techniques that enumerate traditional indicators of faecal contamination (such as enterococci and faecal coliforms) (Leclerc *et al.* 2001). In spite of their widespread acceptance, many studies have raised questions about the use of current indicators as predictors of health risks. This is particularly relevant to viral contamination of shellfish, since it is possible that shellfish that meet *E. coli* standards for human consumption may still contain human enteric viruses that cause gastroenteritis and hepatitis (Doré *et al.* 2000). One possibility is to target pathogens instead of indicators. For years this has been a nearly impossible task, but recent advances in molecular biology (including PCR-based technologies) are allowing microbiologists to monitor multiple targets simultaneously without the need to culture the pathogens. This is relevant to source identification as several host-specific PCR-based methods are currently available and several more are likely to emerge in the near future.

While some of the problems associated with conventional methods can be addressed with molecular techniques, many developing countries may not have access to the type of equipment needed for state-of-the-art molecular analyses. In addition, the limited number of molecular laboratories and lack of training opportunities suggest that technology transfer and subsequent use of some molecular-based techniques for source identification (RFLP, AFLP) may be slow to develop in some countries. Therefore, many of the most sensitive and advanced discriminatory MST methods may not be available soon for source identification in developing countries. However, relatively inexpensive and

simple methods like ARA and CUP, coupled with monitoring strategies like targeted sampling, may be viable options for source identification in developing countries as these methods rely on technologies that are readily available in most microbiology laboratories. Once some of the primary faecal sources have been identified, molecular assays could be used as supporting tests. Where antibiotic use differs between developed and developing countries in fields such as medicine, agriculture and aquaculture, one might expect a need to adapt the antibiotic resistance analysis strategy accordingly. To date, there has been no standardized approach for selecting antibiotics (and concentrations) for antibiotic resistance analysis, even in developed countries where the method has most commonly been applied. One additional advantage of applying ARA for source tracking is that it may also be possible to collect data for antibiotic resistance surveillance purposes at the same time. This an important fact as there is growing concern about the spread of antibiotic resistance in food and waterborne bacteria (White *et al.* 2001; WHO 2001; Summers 2002).

One of the most significant concerns about water pollution is the presence of human pathogens in source waters used for drinking, recreation and food production. This is an important concern to the shellfish industry as shellfish are capable of concentrating significant amounts of pathogenic agents that are commonly implicated in food outbreaks (Koopmans *et al.* 2002; Yeung and Boor 2004). Most of the reported outbreaks linked to shellfish involve human enteric viruses like caliciviruses and hepatitis A (Potasman *et al.* 2002). Due to the economic impact of shellfish bed closures and documented public health risks, detecting and reducing human faecal sources is relevant to local and federal governments. Leaking septic tanks and inadequate municipal wastewater treatment are often a source of human faecal pollution and therefore the ability to trace faecal pollution to these sources is a high priority. Several human-specific LIMs have been reported in the literature and these could prove to be valuable tools to track human sources of pollution in both developed and developing countries. However, the incidence of zoonotic outbreaks in recent years also underscores the need for tools to identify animal faecal sources, particularly in areas of poor sanitation and coexistence of high numbers of human and animals.

5.7 CONCLUSIONS: CURRENT NEEDS AND FUTURE DIRECTIONS IN SOURCE IDENTIFICATION

A good understanding and formulation of the nature of the faecal contamination problem is required before selecting an appropriate method and conducting a source tracking study. It should be recognized that MST studies can be

expensive and time-consuming, and the current state of the science may not be able to resolve complex multi-host discrimination questions over large temporal and spatial scales. In some cases though, it may be possible to break down large source tracking problems into more manageable studies through a targeted sampling approach as described earlier in this chapter.

In any MST study, it is advisable to have multiple lines of evidence to make inferences about faecal sources. Additional lines of evidence could come from local knowledge of nearby faecal sources, sanitary survey information or observations of animal populations and faecal droppings. It is also possible to supplement MST results with information from other types of source tracking methods. In addition to microbial methods, there are also chemical methods for source tracking (such as fluoremetry) and novel applications of molecular methods for the detection of eukaryotic mitochondrial DNA (Martellini *et al.* 2005; Caldwell *et al.* 2007) that may be useful. Again, careful consideration of the faecal contamination problem, budget size, availability of equipment and expertise are factors that have an impact in designing a practical MST study.

It should be noted that many shellfish areas occur in estuary and marine environments where MST methods have been less studied. Environments such as estuaries present significant challenges for MST studies since they can occur at the confluence of diverse marine and land-based faecal pollution sources, and they may present complicated mixing zones and salinity gradients for freshwater run-off and marine tidal fluxes. The choice of a suitable source identifier organism to use in these situations needs further study. For example, how source identifier organisms survive as they transfer from freshwater into marine waters can have an impact on the ability to detect land-based faecal pollution sources. Additional knowledge about the ecology of source identifier organisms like *E. coli*, enterococci, and *Bacteriodes* spp. in estuarine and marine environments would enhance confidence in the results of MST studies.

Since much of the public health risk associated with shellfish has been related to viral and protozoan contamination, a greater effort to develop and test LIMs based upon viral and protozoan source tracking markers would seem to be warranted. The conventional source identifier microorganisms chosen to date in temperate freshwater source tracking studies (including coliform bacteria like *E. coli*) may be less useful for shellfish contamination source tracking problems in tropical marine coastal waters. For example, Doré *et al.* (2000) indicate that shellfish that meet *E. coli* standards for human consumption may contain human enteric viruses that cause gastroenteritis and hepatitis. Gomez-Bautista *et al.* (2000) raised concern about shellfish monitoring programs being conducted with respect only to coliform bacteria contamination and not for the *Cryptosporidium* spp. oocyst contamination they were finding in shellfish. Source tracking

methods based on the alternate microbial markers may have added value in being more closely associated with public health risk.

In the near future, more host-specific genetic markers are expected to emerge as the result of ongoing microbial genome sequencing projects. It is also expected that novel markers will be developed using gut metagenomic approaches (Handelsman 2004). The latter approaches will offer the opportunity of discovering genes that are involved in host-microbial interactions, which in turn could prove extremely valuable in the development of host-specific genetic markers. A significant increase in the number of host-specific markers would likely translate into the preferential use of nonculture-based LIMs in the years to come. Moreover, the availability of additional markers will allow scientists to challenge samples with multiple markers, increasing the discriminatory power of LIMs. Many of the emerging methods will be based on relatively simple real-time PCR methods (also known as quantitative polymerase chain reaction or qPCR). These methods have the potential of quantifying the relative levels of different sources present in hundreds of water samples in a rapid fashion. Other methods could rely on the simultaneous detection of multiple host-specific markers using a microarray or biochip platform. While microarray technology is currently limited by detection sensitivity for identifying low-abundance markers in complex microbial communities, applications for comprehensive genotyping of individual isolates shows promise. For example, microarrays have already been applied to characterize virulence genes in *E. coli* isolates (Bekal *et al*. 2003). The development of biochips that include genes specific to indicators of faecal pollution, pathogens, and source tracking markers will emerge as tools for monitoring microbial water quality and for microbial risk assessment analyses. However, the successful application of many of these methods in field studies will depend in part on the robustness of the sequencing databases, their validation against non-target faecal sources, and the occurrence and survival of targeted microbial populations outside of the gut environment.

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6

Components of microbiological monitoring programmes

R. Lee and L. Murray

Microbiological monitoring of bivalve mollusc production areas by national or regional authorities is usually undertaken as part of a programme to classify the areas according to the perceived risk of the presence of pathogenic micro-organisms based on the concentrations of faecal indicator bacteria present in the area.

The indicator bacteria used are generally of the coliform group: total coliforms, faecal coliforms or *E. coli* depending on the requirements of the legislation which applies. Although a number of other faecal indicator bacteria have been used for other programmes (e.g. drinking or recreational waters) these have not generally been applied to the classification of shellfish harvesting areas.

Figure 6.1 shows the proportion of samples positive for *Salmonella* spp. in different classes of shellfish harvesting areas in England and Wales. Other workers have showed a similar change in the occurrence of norovirus positives with
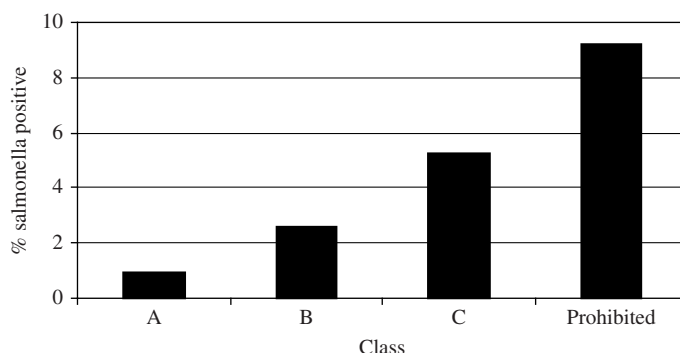
Figure 6.1. Percentage of samples in each European Union classification category positive for *Salmonella* (data from England and Wales).
Source: Lee and Younger, 2003.

class. The classification system determines whether the area can be used for production at all and, if so, the level of treatment (relay, depuration, heat-treatment) which needs to be applied to harvested bivalves prior to sale for consumption.

There are two principal systems in use in the world. One is that of the European Union (EU), under Regulation (EC) No 854/2004 (European Communities 2004). This system uses the monitoring of *E. coli* in the shellfish flesh and intravalvular fluid. The criteria for classification of areas under the European system are summarized in Table 6.1. The EU Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve molluscs has published a Good Practice Guide for the Microbiological Monitoring of Bivalve Mollusc Production Areas which provides recommendations on the application of the requirements of the Regulations (Anon 2007). The other is the United States (US) National Shellfish Sanitation Programme (NSSP) which uses monitoring of water samples from the production area (US FDA 2008). The criteria for classification of areas under the NSSP are summarized in Table 6.2. Fuller details may be found in the references. Other countries which trade with Europe or the United States will adopt one or both of the systems depending on the circumstances. Some countries have produced hybrid systems as a practical approach to trading with both blocks.

The general principles of microbiological monitoring programmes will be described below and reference will only be made to specific examples to illustrate practical details. While some systems still include the possibility of using total coliforms as indicators of harvesting area quality, this section will address aspects of monitoring programmes relating to the use of faecal coliforms, and more particularly *E. coli*, as more specific indicators of faecal contamination.

Table 6.1 EU criteria for the classification of shellfish harvesting areas

Class[1]	Microbiological standard[2]	Post-harvest treatment required
A	Live bivalve molluscs from these areas must not exceed 230 MPN *E. coli* per 100 g of flesh and intra-valvular liquid[3].	None
B	Live bivalve molluscs from these areas must not exceed, in 90% of the samples, 4600 *E. coli* per 100 g of flesh and intravalvular liquid. In the remaining 10% of samples, live bivalve molluscs must not exceed 46 000 *E. coli* per 100 g of flesh and intravalvular liquid[4].	Purification, relaying or cooking by an approved method.
C	Live bivalve molluscs from these areas must not exceed the limits of a five-tube, three dilution MPN test of 46 000 *E. coli* per 100 g of flesh and intravalvular liquid[5].	Relaying or cooking by an approved method.

MPN – most probable number.
[1] The competent authority has the power to prohibit any production and harvesting of bivalve molluscs in areas considered unsuitable for health reasons.
[2] The reference method is given as ISO 16649-3.
[3] By cross-reference from Regulation (EC) No 854/2004, via Regulation (EC) No 853/2004, to Regulation (EC) No 2073/2005.
[4] From Regulation (EC) No 1021/2008.
[5] From Regulation (EC) No 854/2004.

6.1 SAMPLE MATRIX – SEAWATER OR SHELLFISH?

As noted above, there are two broad approaches to monitoring the microbiological quality of shellfish harvesting areas, using either seawater or the shellfish themselves.

6.1.1 Seawater

This has the advantage that the matrix is relatively easy to sample and analyse, permitting larger quantities of data to be assembled at relatively little cost. There is also the advantage that it avoids the complication of different species of shellfish yielding different results (see below). However, there are a number of other considerations. For example, the depth at which the water samples are taken may affect results as stratification effects may influence the microbiological relationship between surface water and benthic shellfish. There is also a time delay between the occurrence of contamination and its clearance from the water column and the same effect being seen in shellfish, which depends on the

Table 6.2 NSSP criteria for the classification of shellfish harvesting areas

Classification	Total coliforms per 100 ml water		Faecal coliforms per 100 ml water		Treatment required
	Geometric Mean	90% compliance[1]	Geometric mean	90% compliance[1]	
Approved areas[2]	≤70	≤230	<14	<43	None
Restricted areas[3]	≤700	≤2300	≤88	≤260	Purification or relaying in an approved area
Prohibited areas	No sanitary survey or conditions for approved/ restricted areas not met[4]				Harvesting not permitted

[1] Values for 5-tube decimal dilution test – different 90% compliance levels are given for the 3-tube MPN and mTEC membrane filtration tests.
[2] Determination of approved area status must be based on a minimum of 15 samples from each monitoring station.
[3] 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.
[4] Considerations other than the concentration of contaminants may be used to declare an area prohibited.

dynamics of uptake and depuration of microbes by the shellfish. In general, contamination effects will be seen for a longer period of time in the shellfish than in the water column.

6.1.2 Shellfish

Sampling shellfish provides a direct estimate of the level of their microbiological contamination and avoids some of the problems identified above with sampling of seawater. However, shellfish are much more difficult to sample, transport and test than is seawater and this imposes practical constraints and increases costs. Another problem with using shellfish is that different species co-existing in the same area will tend to show different levels of contamination (see below) and thus, unless an indicator species is used, it is necessary to sample all commercially important species.

Lart and Hudson (1993) investigated the differences in *E. coli* concentrations in several species of shellfish sampled in the same geographical location. They detected significant differences between species but these varied depending on the season. The work included only a small number of sampling occasions and thus the results would have been complicated by the other factors affecting individual

E. coli concentrations in bivalves. Monitoring programmes in England and Wales show a general tendency for the degree of contamination to be in the order (from highest to lowest):

   i. Mussels (*Mytilus edulis*); flat oysters (*Ostrea edulis*); Manila clams (*Tapes philippinarum*);
   ii. Pacific oysters (*Crassostrea gigas*);
   iii. other clams, including razor clams (*Ensis* spp.).

The relative *E. coli* content of cockles (*Cerastoderma edule*) appears to vary with location and may reflect the nature of the seabed substratum amongst other factors.

The observed species differences probably reflect the interaction of a number of different factors including their biological activity (rate of uptake and depuration) and location in the water column/substrate. The biological activity will also be affected by season, water temperature and salinity. Low salinities may result in some species stopping their filtration activity altogether and this may mean that they are not exposed to the full contamination effects of rainfall-associated contamination episodes.

The factors described above will affect the detection of contamination episodes in different species. For example, following a breakdown at a sewage works, mussels will normally show a quick increase, followed by decrease, in contamination whereas the event will affect *E. coli* levels in Pacific oysters more slowly and the levels in these will remain higher for longer after the contamination event is over.

There is little information as to whether the differences observed in *E. coli* contamination of different bivalve species reflect differences in likely pathogen content. Although aspects relating to differences in biological activity might be expected to affect indicators and pathogens similarly, the markedly different depuration rates for bacterial indicators and viral pathogens may affect any such relationship (Lees 2000).

6.1.3 Seawater and shellfish

A number of shellfish monitoring programmes, such as those in Canada and New Zealand, essentially consist of a hybrid between seawater and shellfish monitoring. Under such systems, water monitoring may yield the basal classification for an area but testing of shellfish themselves may be undertaken in response to contamination events or failures of specified faecal indicator levels in incoming shellfish at depuration or packing plants. In such cases, re-opening of harvesting areas may be based on a return of indicator bacteria in the shellfish to predefined levels.

6.1.4 Seawater and shellfish equivalence

An EU Working Group undertook a statistical study of *E. coli* results from a large number of paired shellfish and seawater samples from a number of sites in several countries to determine whether a relationship existed between them (European Commission 1996). This showed that there was a weak relationship between the raw data but a much stronger relationship between the paired geometric mean data from each sampled site. This was taken to indicate that there was a relationship between the general level of contamination in seawater and shellfish at each site. It was concluded from the results of the analyses that the USA and EU requirements for approved and class A areas, and restricted and class B areas, respectively were equivalent.

The United States Food and Drug Administration (US FDA) used a semi-parametric statistical approach to analyse data from a combined EU and United States data set. The analyses were undertaken on the whole data set and differences between sites were not taken into account. On the basis of these analyses, the FDA concluded that the requirements of the United States categories were tighter than the corresponding EU categories (W Burkhardt, personal communication).

One difference between the two studies was that the EU analysis assumed that the geometric mean water concentration was the overriding factor in determining classification status under the NSSP while the FDA study assumed that the 90 percentile was principal factor in this regard.

In countries where hybrid systems are used, it has been reported that exceedences of the stipulated levels for shellfish flesh may occur despite continued compliance with the requirements for seawater (Campbell 2004). This indicates that a system of monitoring solely based on seawater may not be adequately protective of public health.

6.2 SAMPLING PLANS

The results obtained in a microbiological monitoring programme, and thus the resulting classification, will depend on the design and implementation of the programme. The three principal factors shown to affect results are the sample that is taken, the location of sampling points (primarily in relation to sources of contamination) and the frequency of sampling. The sampling plan should depend on the outcome of the sanitary survey (see chapter 8) together with an understanding of the effects of environmental factors. The following need to be specified:

- type of sample (matrix: seawater or shellfish; shellfish: species);
- sample site location (and latitude allowed around the defined point);

- number of samples to be taken per sample type per year;
- randomised or targeted sampling programme;
- depth, tidal state, other environmental factors;
- action to be taken (if any) in the event of missed, lost samples; and
- action to be taken (if any) in the event of samples exceeding the criteria for the class of area.

6.2.1 Spatial effects

Spatial effects include two dimensional considerations (geographical location) and also depth, including position in relation to the seabed material. Lart and Hudson (1993) studied *E. coli* concentrations at regular points in grids with overall dimensions varying from tens of metres to 1.5 kilometres. The grids were located in different harvesting areas. Significant differences in mean *E. coli* content were detected on scales as small as metres but the effect varied between harvesting areas. In some, but not all cases, the significant differences could be related to physical factors such as distance from presumed contamination sources.

Belliaeff and Cochard (1995) undertook a detailed study of spatial variation across a French mussel farming area (approximate dimensions 6.5 Km × 0.9 Km) on two separate sampling occasions. They found significant variation in faecal coliform concentrations across the area. They concluded that attempts to obtain an overall picture of spatial contamination across a bed may be prohibitive in terms of cost and suggested that a practical solution would be to restrict sampling to the points representing the highest level of faecal contamination.

Younger *et al.* (2003) noted that variations were often found in the extent of *E. coli* contamination seen in mussels along the length of cultivation ropes and this is assumed to be the result of contamination being confined to particular depths due to density gradients. Some effects may also result from the resuspension of contaminated sediment and differences in the extent of contamination may be seen in the same species grown on the seabed and on trestles in the same area.

In practice, the number of sampling points, located to detect the potential impact of the contamination sources revealed by the sanitary survey, may need to be relatively large during the initial bacteriological survey and early stages of classification. The number may be able to be reduced as data is accumulated and reviewed.

6.2.2 Temporal effects

The effect of sample time on *E. coli* concentrations is largely influenced by a number of the factors discussed in section 6.1.2, together with the influences of

spring/neap and high/low tidal cycles. These factors combine to yield the possibility for *E. coli* concentrations to vary by up to approximately 10^4 per 100 g over a period of hours (Tattersall *et al*. 2003; Younger *et al*. 2003). The magnitude of the variation will depend on the level of contamination in the area, as well as the effects of the environmental and biological factors. The difference in time that may be found between peak *E. coli* concentrations in different species following a contamination event was noted above. There is not necessarily any direct relationship between the *E. coli* concentration in an individual sample and the likelihood of the presence of a viral pathogen in the harvesting area (Lees 2000). It is therefore not possible to use individual *E. coli* results to predict the level of contamination over even a period as short as one day and this invalidates the use of small numbers of results to determine short-term changes in classification status.

6.2.3 Randomised or targeted sampling

One or other of these two approaches is normally taken to the design of sampling plans. Randomised sampling is intended to reflect the overall contamination status of an area while targeted sampling is intended to concentrate on those conditions deemed likely to yield the highest results. The latter should be more protective of public health.

Both approaches can be difficult to properly implement in practice. Bad weather may prevent fieldwork at times when sampling has been scheduled. Particularly when shellfish are monitored, it may not be possible to sample at certain states of tide, such as low tide when sampling by boat or high tide when sampling by hand-picking. With targeted sampling, a detailed analysis has to be undertaken with respect to the various environmental factors that may affect the results and sufficient data may not be available to achieve this.

In the EU, neither approach is explicitly identified in the Directive but it is usually assumed that randomised sampling is intended and many Member States try to achieve this as far as possible. In the US NSSP, both approaches are considered.

6.3 SAMPLING METHODS AND SAMPLE TRANSPORT

There is a need to standardize sampling and sample transport procedures in order to ensure that samples that are analysed are representative of the sample point. The following need to be specified:

- the place and type of sample;
- the means of sampling;

- sampling record (perhaps on sample submission form);
- sample containers and outer packaging to be used;
- temperature control during transportation; and
- acceptable time lag between sampling and analysis.

The place and type of sample will derive from the sampling plan, as may also the sample time. For waters, grab samples will normally be taken from a specified depth beneath the surface. For bivalve molluscs, it is advantageous where possible to sample using the means normally used for commercial harvesting as additional contamination may be introduced during some dredging procedures. Where this is not possible, or where an indicator species is being used, samples may be taken by other means (including hand-picked) or bagged shellfish may be kept at the monitoring point for the purpose of sampling. With the latter, the effect of location in the water column should be considered. This is also important when sampling seawater.

Faecal coliforms and *E. coli* do not tend to multiply in seawater or shellfish samples stored at 10°C or less (Cook and Ruple 1989; Lart and Hudson 1993). Prolonged storage at low temperatures may result in reductions in counts and it is recommended that samples are stored (whether in transport or otherwise) at a temperature of less than 8°C and that the maximum time lag between sampling and analysis is 24 hours. Properly packed cool boxes containing ice packs (not in direct contact with the samples) should achieve a temperature less than 8°C within four hours and maintain this for at least 24 hours. Samples should not be frozen as this will result in a marked decrease in faecal coliforms/*E. coli* concentrations.

Sampling officers should be provided with a protocol containing details as to how samples should be taken, cleaned of sediment (for shellfish), packed and transported. Where samples are taken with the help of the industry, for example if an official boat is not available, it is preferable for this to be done under the supervision of a sampling officer. If this is not possible, sampling protocols and relevant training should be provided and audits undertaken to ensure compliance with the protocol.

6.4 MICROBIOLOGICAL TESTING

It is essential that the microbiological tests methods used in the monitoring programmes are not too inhibitory to bacterial cells stressed by exposure to the marine environment. The limits of detection and quantification for any chosen test are important points for consideration – the chosen test must be able to yield a numerical result lower than the regulatory limit. In the case of shellfish this is

normally <230 *E. coli*/100g for shellfish being sold for human consumption without prior treatment. The limit of quantification will be determined by a combination of both the theoretical properties of the test method and the sensitivity of the media chosen.

The most commonly used techniques for the enumeration of faecal coliforms and *E. coli* in seawater from shellfish harvesting areas are the Most Probable Number Technique (MPN) and membrane filtration. For the MPN method, the first stage medium is commonly lauryl tryptose broth or modified minerals glutamate broth and positive tubes are confirmed as either faecal coliforms or *E. coli*, as required, by appropriate methods. The US NSSP also includes a procedure using A-1 medium which yields results in 24 hours (note: this method is not suitable for use with shellfish). For the membrane filtration method, either a membrane lauryl sulphate broth (MLSB)-soaked pad or mTEC agar, followed by confirmation is used. Details of appropriate methods are given by the Environment Agency (EA 2000) and the American Public Health Association (APHA 1970).

The techniques most commonly used for the enumeration of faecal coliforms and *E.coli* and in shellfish are MPN, direct counting on agar plates and impedance techniques such as the Malthus and BacTrac. Within each of these enumeration techniques there are variations both in terms of the media that may be used and the precise format of the method used under the technique itself. Within Europe, ISO TS 16649-3 (based on Donovan *et al*. 1988) is the reference method for the testing of shellfish for *E. coli*. This method is a two-stage; five tube by three dilution MPN. The first stage of the method is a resuscitation requiring inoculation of minerals modified glutamate broth (MMGB) with a series of diluted shellfish homogenates and incubation at $37\pm1^\circ$C for 24 ± 2 hours. The presence of *E. coli* is subsequently confirmed by subculturing acid producing tubes onto agar containing 5-bromo-4-chloro-3-indoly-ß-D glucuronide and detecting growth on the tryptone bile glucuronide agar (TBGA). In France, a significant proportion of samples from the microbiological monitoring programme are tested using an impedance procedure which has been validated against an MPN method (Dupont *et al*. 2004). It is recognized that plate count methods may not provide the sensitivity needed for assessing compliance with current legislative requirements. It is also the case that lower recovery of stressed cells may occur on solid media than in appropriate liquid media.

6.4.1 Validation of alternative methods

Where reference methods are specified within the framework of a shellfish sanitation programme (in legislation or guidance), these should either be used for the monitoring or alternative methods should be validated against them

according to scientifically accepted criteria. There are a number of published procedures including:

(1) International Standard ISO 16140, Microbiology of food and animal feeding stuffs- Protocol for the validation of alternative methods;

(2) AOAC International (1999) Qualitative and Quantitative Microbiology Guidelines for Methods Validation. Journal of AOAC International, 82 (2), 402–416; and

(3) NordVal Validation protocol, Protocol for the validation of alternative microbial methods. NV-DOC. D – 2004-01-01.

6.4.2 Accreditation and proficiency testing

Accreditation ensures that laboratories undertaking testing for specific purposes, for example as part of an official control programme, achieve at least a minimum standard with respect to the control of internal procedures and the performance of analytical tests. This may be achieved via a national accreditation body, inspection by the competent authority, or both. Details of accreditation procedures can be found in ISO 17025 (ISO 1999) while those for the evaluation of laboratories under the US NSSP can be found in the model ordinance (US FDA 2008).

Participation in relevant proficiency testing schemes also enhances equivalency of testing. Both the US FDA and the UK Health Protection Agency run schemes that are specific for shellfish. The FDA scheme includes samples for the water test as well as shellfish. It is important that any proficiency-testing scheme includes a formal scoring and score review scheme with advice being offered to poor performers. As the consequences of misclassification are significant from both the public health and commercial dimensions, it is necessary that persistent poor performers do not continue to take part in official microbiological monitoring programmes until the poor performance issue has been addressed.

6.5 INTERPRETATION OF MONITORING PROGRAMME DATA

6.5.1 Time series data sets

Due to temporal variation in the concentration of faecal indicator bacteria, and the fact that the presence of pathogens may generally not be related to the concentration of faecal indicator bacteria in single samples, assignment of risk status (classification) of harvesting areas is normally based on an assessment of data over a period of time. It is also necessary to establish the minimum number of results to be considered over that period. Both of these need to be specified

in advance so that they are considered in establishing the monitoring plans. The microbiological status for a future period and, thus, the treatment to which the shellfish have to be subjected, is based on historical data and this makes it essential that a scientifically valid approach is taken to the interpretation of the data.

It is generally accepted that the minimum useful monitoring frequency for *E. coli* in shellfish is monthly and that the minimum review period should be one year. However, general differences in *E. coli* results may be seen between dry and wet years in some areas and a longer term review period may help to reduce this variation – some programmes currently include a three-year review period. There is also a need to review data when there is a known change in contaminating inputs to a harvesting area (such as sewage improvement schemes). However, there will usually be a lag before sufficient data is accumulated to convincingly show the effect of such changes. Where a long period of monitoring has shown that the microbiological status of an area is stable, a risk assessment may be undertaken to justify a lower sampling frequency. It must be borne in mind that this will reduce the likelihood of detecting unpredicted additional sources of contamination impacting on the harvesting area.

Certain contamination events may be deemed extremely unlikely to recur and it may be considered valid to disregard any microbiological results associated with these in considering the longer-term status of an area. These may include exceptional rainfall events (for example with a one in five year, or greater, return period) and sewage treatment plant or sewerage breakdowns (see below) where long-term remedial work has been undertaken.

Repeat samples undertaken as part of an investigation into abnormally high results from an area should not be included in the analysis of the long-term status of an area, due to potential introduction of bias, but they may form part of a risk assessment of the short-term status of an area (see below).

6.5.2 Analytical tolerance

Laboratories undertaking accredited testing increasingly have to identify the uncertainty associated with their analyses in order to satisfy the accreditation bodies. A technical specification for the determination of uncertainty with respect to microbiological testing has been developed within the International Organization for Standardization (ISO) (ISO/TS 19036:2006). At the moment, the determination of microbiological uncertainty concentrates on the matter of variability of results and the question of bias of methods is being ignored. In order to reduce the effect of bias as much as possible, it is important that reference methods are precisely defined, any alternative methods are

properly validated and accreditation and proficiency testing are undertaken as described above.

While accreditation bodies may expect laboratories to report the variability component to the customer, there is little advice as to how this should be interpreted by the end-user. The matter is made especially difficult because many microbiological standards, including many of those for harvesting area classifications, have been derived without explicitly considering the application of uncertainty. It is therefore not clear as to whether any allowance for variability should be biased towards the protection of the consumer or to the benefit of the shellfish industry. Given the public health intent of shellfish sanitation programmes, it could be viewed that the former approach should be taken. With an existing standard, changing from a situation where analytical uncertainty is not taken into account to one where it is will change the average level of microbiological contamination allowed in the area, with consequent change to the public health risk. Within the EU, it has been decided not to progress the application of analytical uncertainty to microbiological criteria at the present time.

The 90% compliance values for the MPN procedure in the NSSP classification criteria were derived from the specified geometric mean values largely on the basis of the analytical variability inherent in the MPN test. As any environmental variability is additional to this and is often much larger, the 90% compliance values tend to be the principal factor in determining the classification status of areas.

Within the EU, some Member States allow a certain tolerance of results to be above the limits given in the Directive, usually justified on the basis of either analytical tolerance or environmental variability. Changing the percentage compliance will change the implied underlying microbiological status of the harvesting area and the consequence with regard to pathogen occurrence is illustrated in Figure 6.2.

6.6 MONITORING IN RELATION TO POLLUTION AND ILLNESS EVENTS

Monitoring for faecal pollution indicators should be considered in the case of exceptional contamination events, such as a sewage treatment plant or sewerage failure, or when cases of illness have been attributed to shellfish harvested from a particular area. High results will inform a risk management strategy for an area. On the contrary, for reasons already discussed, low results may simply mean that the faecal indicator bacteria are no longer present while pathogens, particularly viruses, may still be present in the shellfish. Any period
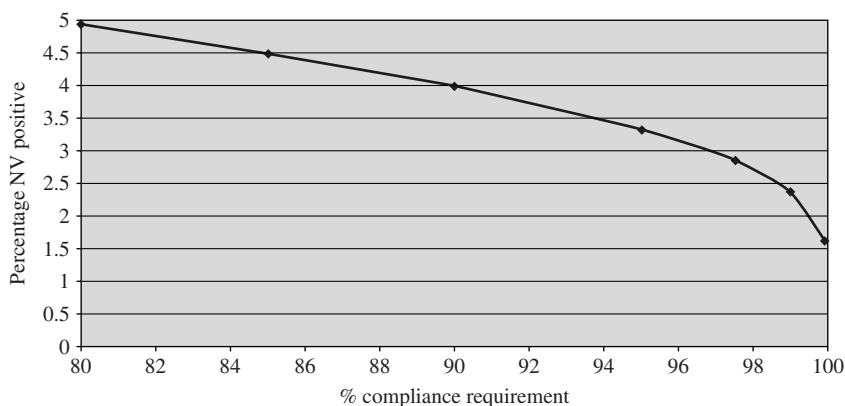
Figure 6.2. Change in predicted likelihood of a viral pathogen (Norovirus) with change in percentage compliance with the EU class A requirement of 230 *E. coli* per 100g shellfish.
Source: an analysis undertaken by the European CRL for bacteriological and viral contamination of bivalve molluscs based on data from an EU-funded research project.

of closure should be based on an assessment of all available information, not just that from any monitoring, and should consider the time needed for shellfish to clear viral pathogens.

6.7 MONITORING FOR PATHOGENS

Most harvesting area monitoring programmes are based on the use of faecal indicators as described above. However, for human pathogenic vibrios, where there is known to be no correlation between such indicators and the pathogens, a number of programmes incorporate direct monitoring for the pathogens themselves. On both a research and an investigative basis, this has been extended to some of the water quality-related illnesses such as norovirus and hepatitis A. Lee and Kay (2005) identified the following scenarios where such direct pathogen monitoring might be appropriate, namely:

- investigation of bivalve mollusc-associated outbreaks of illness;
- in support of the development of risk assessments;
- investigation of the impact and persistence of contamination events (e.g. in the event of sewage treatment work or sewerage system malfunction or breakdown);

- validation of current monitoring programmes based on faecal indicator organisms (to support decisions on programme content and data interpretation); and
- secondary monitoring to supplement programmes based on faecal indicator organisms.

However, they also expressed some reservations in introducing such monitoring without further investigation of the following considerations:

- faecal indicators yield a general measure of the risk of contamination;
- individual pathogens may not be present when such a general risk of contamination exists but other pathogens may be present;
- it is not presently practical to monitor for all possible pathogens;
- new or emerging pathogens may not be detected (including new variants of highly variable viruses); and
- shellfish-associated bacterial infections still need to be considered.

Molecular methods also do not yield a direct assessment of viability and the significance of results obtained using such methods are not always clear. A considerable amount of practical knowledge has been built up over the years with respect to the use of faecal indicators in monitoring programmes and it is important that similar information is obtained with regard to pathogen monitoring in order to prevent its potential misapplication.

6.8 INTERACTION WITH MITIGATION STRATEGIES

The results of the microbiological monitoring programmes dictate controls or mitigation measures that are applied to reduce the risk of illness associated with shellfish consumption. The general requirements are given in Tables 6.1 and 6.2. The principles of harvesting area controls (including short-term closures) are discussed further in chapter 10 while methods used to reduce the risk from moderately contaminated shellfish (heat treatment, relay and depuration) are discussed in chapter 9.

6.9 INTERACTION WITH OTHER MONITORING STRATEGIES

Some harvesting area programmes also use surrogate factors such as rainfall or river flow, where these are known to be associated with high microbiological loads, to trigger harvesting restrictions or other controls. The use of these factors

is discussed further in chapter 10. It is important that the use of such surrogates is based on an explicit evaluation of the risk of microbiological contamination (which implies statistical evaluation of microbiological data) and that contamination cannot occur at other times when the surrogate does not trigger controls. Inevitably, although there is increasing interest in the use of such strategies, these will not replace the need for microbiological monitoring, whether based on the use of faecal indicators, direct detection of pathogens or a hybrid of the two.

6.10 CONCLUSIONS – RESEARCH GAPS AND FUTURE CHALLENGES

There are a number of research gaps and future challenges. These are:

- A requirement for progress in the identification of an umbrella system which will accommodate the various approaches currently used around the world for microbiological monitoring and subsequent data analysis leading to risk assessment (in the broadest sense). Such a system needs to be based on sound science and the principle of public health protection.
- A need to assess how well the separate approaches of seawater and shellfish flesh monitoring for indicators relates to the risk of pathogens being present in the shellfish themselves.
- A need to reassess whether the presently used indicator organisms are the best for such assignment of risk and to evaluate whether others may be more suitable. Considerable work has been undertaken on F(+)-coliphage with regard to prediction of the presence of viral pathogens (Doré *et al*. 2000) but further work is required to determine whether this, or other potential indicators, perform adequately in this regard on a global basis.
- With both alternative indicators, and direct monitoring for pathogens, there is a need to determine the effect of sampling, sample transport and analytical procedures, as well as the magnitude of spatial and temporal variation.
- With regard to the possible use of more active management of shellfisheries, and the use of surrogate variables (such as river flow) in programmes, more research is required to identify how well both conventional and surrogate indicators predict the presence of all faecally-derived pathogens and how long the pathogens may persist after the levels of the indicators return to normal.

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7

Real-time monitoring technologies for indicator bacteria and pathogens in shellfish and shellfish harvesting waters

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The counting of bacteria in water has been a critical element in protecting public health in the last century. In the early part of the century scientists were able to grow many species of bacteria and differentiate them from each other using biochemical tests. They also had observed that certain bacteria were always found in the faeces of humans and other warm-blooded animals and that significant disease was also associated with faecal wastes. This association was recognized early on by the scientists who were the fore-runners of our present-day public health scientists. A bacterium originally called *Bacillus coli*

was found in high numbers in the faeces of an infant and shortly thereafter was found in the faeces of healthy humans and warm-blooded animals (Escherich 1885). Pathogens, such as the microorganism that causes cholera, were difficult to grow with the nutrient media available at the time. *B. coli*, on the other hand, grew on simple media and was easily detected in water. The specific identification of *B. coli*., however, was not easy and microbiologists were soon isolating all of the bacteria that looked like and behaved like *B. coli*. This resulted in the group name coliform, meaning having the form of a *B. coli*. Soon, glucose was replaced in coliform media with lactose. The ability of coliforms to specifically metabolize lactose and produce acid and hydrogen gas as end-products allowed this group of organisms to be easily identified.

Dunham (1898) recognized the value of gas production for detecting coliforms and used this characteristic to identify the presence of coliforms in liquid culture. He simply inverted a small tube and placed it in the culture tube. This tube captured the gas produced by coliforms. Phelps (1907) developed a coliform index which used utilized gas production. The index was based on a dilution concept in which the reciprocal of the highest dilution where growth and gas production occurred was reported as the best estimate of the coliform density in a given volume of water.

McCrady (1915) was the first to utilize statistical probability theory to estimate numbers of coliform bacteria using the fermentation tube method. The technique, based on the number of tubes showing growth and gas production, provided a most probable number (MPN) estimate of the number of coliform bacteria in a particular volume of sample (McCrady 1915). However, solid media, to which water samples can be applied, were preferable to MPN methods because colonies growing on the surface of the medium could be counted rather than estimated. Initially the small volumes of sample that must be used with solid agar media precluded their use with samples that contain small numbers of the bacteria being counted. This problem was solved with the introduction of the membrane filter which allowed larger volumes of water to be analyzed. 100 ml or more of sample can be passed through the membrane filter, which can then be placed on a selective, solid medium and incubated for a selected time period, after which colonies on the filter can be identified and counted.

The common factor between these two quantitative approaches to counting bacteria is the requirement for cell multiplication to occur over a sufficient period of time, so they can be easily observed. In both cases the time to colony formation is between 20 and 24 hours. Compressing this time interval has been the goal of water microbiologists for many years. In one case this was accomplished using a membrane filter technique and a nutrient medium that

maximized the cell doubling time which allowed microscopic identification of the colonies in about seven hours (Reasoner *et al.* 1979). This is about the lowest time limit for obtaining results with culture methods for quantifying bacterial indicators in water samples. However, there is a new generation of instruments and techniques being developed to quantify indicator bacteria and pathogens that will provide monitoring results in a much shorter period of time.

Molecular methods are at the forefront of these new technologies. The advent of the polymerase chain reaction (PCR) technique (Mullis and Faloona 1987) holds great promise as a rapid method for measuring environmental water quality. The PCR was a significant step in the development of highly specific, rapid methods for identifying microbes associated with faeces. It did have drawbacks, however, in that the original post-amplification procedures were time consuming and did not lend themselves to quantification. In the mid-1990's, the real-time quantitative PCR (qPCR) was introduced to the scientific community (Heid *et al.* 1996). This procedure allows both detection and quantification of PCR-amplified nucleic acid sequences without the need for post-amplification procedures. There are numerous thermal cycling instruments available commercially that are capable of rapidly detecting and quantifying microbes in environmental waters by this technique.

Other techniques are also gaining favour in the area of water quality monitoring. Chemical methods that measure adenosine triphosphate (ATP) and enzymatic reactions are also rapid and may be useful for measuring water quality. Antibody based methods that are used with flow cytometry and fibre optic technologies also have some potential, but problems with sensitivity and the small volumes used in these assays are limiting their use. Molecular methods however, are by far the most advanced of the technologies that have been used to quantify microbes used to measure faecal contamination in water.

This chapter will describe some of the available technology for the rapid measurement of water quality and shellfish. Although many high technology methods are described in the literature, very few have been used to test natural samples, such as water samples or shellfish tissue samples. Only methods that have been used to measure natural water samples, whether marine, estuarine or freshwater, or shellfish tissues for faecal indicator bacteria or pathogens will be described in this chapter. The approach will be to describe the procedure in some detail and then briefly review one or two papers from the literature describing how the method has been used to measure indicators or pathogens in samples taken from natural environmental waters or from harvested shellfish. No attempt has been made to provide a comprehensive literature review.

7.1 MOLECULAR APPROACH

The most studied of the new methods for quantifying microbes in water is the qPCR. This technology has many attributes which make it attractive for measuring microbes in water. First, the qPCR method is very specific to the target microbes being detected. Contemporary culture techniques depend on phenotypic characteristics whose presence may be governed by several enzymes that frequently are affected by the physiological state of the microbes. A variable physiological state will result in variable phenotypic characteristics which can at times make identification of the microbe difficult. This variability does not occur with qPCR which detects cells on the basis of specific nucleotide sequences that are unique to the microbes under study. In addition, the qPCR technology is very rapid. Detecting and identifying microbes with cultural methods usually require about 24 hours, the amount of time it takes microbes to grow to the point where growth can be visualized. qPCR results, on the other hand, can be observed in two to three hours, because of the logarithmic amplification of the sequences of interest.

The qPCR process consists of two steps that occur at different temperatures. At a high temperature, double-stranded DNA is denatured to two single strands, completing the first step. At the lower temperature, a number of reactions take place. The first is the hybridization of short pieces of DNA (oligonucleotides) called primers to specific locations on the single strand of DNA. These primers provide a starting point for the synthesis of new double-stranded DNA. A second hybridization involving a highly specific oligonucleotide called a probe, takes place at a point on one of the single-strands of DNA which is between the two primer sites. This probe is unique to the microbe being detected. One of the most commonly used types of probes is called a hydrolysis of Taqman® probe. These probes have a fluorescent reporter dye attached to one end and a quencher dye attached to the other end. When these two dyes remain in close proximity to each other on the probe the reporter dye cannot fluoresce. After the probe attaches to the target sequence, a polymerase begins extending the primer toward the probe, forming new double-stranded DNA. As the extended DNA meets the probe, the probe is cleaved, freeing the reporter dye so that it is no longer in proximity to the quencher dye and can now fluoresce. The formation of double-stranded DNA completely removes the probe from the target sequence allowing the primer extension to continue until a new double-stranded DNA is formed, ending the second step.

These cycles are programmed into a spectrofluorometric thermocycler, which continuously proceeds through the two steps, measuring the amount of fluorescent dye freed in each annealing step. The fluorescent signal intensity is proportional

to the amount of DNA produced. Quantification of the PCR process is measured in terms of the number of two-step cycles and the accumulation of the fluorescent signal to that point where it crosses a baseline and is first detected. The magnitude of the signal generated under a given set of PCR conditions is determined from standard samples of known concentrations used to establish a standard curve.

Quantitative molecular methods for measuring microbes in shellfish have been used to detect both viruses and bacteria. A quantitative reverse transcriptase qPCR method (RT-qPCR) was used by Jothikumar *et al.* (2005) to determine the norovirus density in shellfish meat. The viral RNA from purified shellfish concentrates was recovered by binding to size-fractionated silica after lysis of the viral particles with guanidine isothiocyanates. After elution from the silica particles, the RNA was precipitated in ethanol and sodium acetate. Reverse transcription was then performed using a Geneamp RNA PCR corekit in a Geneamp 9700 PCR system (Applied Biosystems). Finally, Taqman PCR was performed with a QuantiTect probe PCR kit. Compared to other conventional multiplex RT-PCR assays, the Taqman RT-qPCR results were much faster because they do not require additional nested amplification steps.

Vibrio parahaemolyticus is a halophilic, gram-negative bacterium that has frequently been associated with shellfish-associated illness (Rippey 1994). These shellfish-associated outbreaks have stimulated a great interest in the availability of rapid methods for detecting and identifying this microorganism. Ward and Bej (2006) examined multiplex real-time PCR to detect specific genes related to the virulence and species of *V. parahaemolyticus*. They developed the assay using four sets of gene-specific oligonucleotide primers and four corresponding Taqman probes labeled with four different fluorogenic dyes. Ward and Bej used Gulf of Mexico oysters to evaluate their multiplex system. Oyster homogenates were enriched for 24 hours. Following enrichment, DNA was extracted and a small sample of extract was amplified in a thermocycler. Shellfish sample homogenates were seeded with purified genomic DNA of the four genes being amplified as a positive control. Their results showed that 17 of the 34 shellfish were positive for *V. parahaemolyticus* and that four of the positive samples contained a gene indicating that the strain was pathogenic. The other two genes coding for pathogenicity were negative in the 17 samples. This approach will lend itself to delivering timely results in the examination of suspected contaminated oysters or other shellfish meats in outbreak situations.

The use of qPCR to monitor the quality of shellfish harvesting waters has not been considered up to this point in time. However, qPCR has been used to monitor the quality of recreational waters (Haugland *et al.* 2005). Enterococci in beach waters were measured with qPCR and the results, which were obtained in about three to four hours, were shown to have a direct relationship to

gastrointestinal illness in swimmers (Wade *et al.* 2006). The assay is straightforward. A 100 ml water sample is filtered through a polycarbonate filter which is placed in a centrifuge tube containing glass beads. Violent shaking of the beads breaks the cells open, freeing the DNA. The cell debris is sedimented by centrifugation and the supernatant is analyzed by qPCR. This method has also been applied to *Bacterioides* species and it may also have utility in identifying sources of faecal contamination using mammalian species-specific *Bacteroides* spp. strains.

7.2 CHEMILUMINESCENCE APPROACH

The term bioluminescence refers to chemical reactions that occur *in vivo* and which result in the emission of light. If the chemical reaction takes place *in vitro*, the emission of light is termed chemiluminescence. The best known chemiluminescence reaction is the luciferin–luciferase reaction, which has been used for many years to measure ATP from living microbes. ATP can be extracted from living cells and assayed *in vitro* with the luciferin–luciferase system. The resulting emission of light is detected by a luminometer photomultiplier tube. ATP can be measured quantitatively with this system. The amount of light reaching the photomultiplier tube is proportional to the amount the ATP in the sample. Furthermore, the ATP measured should also be proportional to the number of viable cells in the sample.

Chemiluminescence tests that measure the presence of ATP have been used in the food industry, the pharmaceutical industry and the cosmetics industry for many years. Even though this technology has been available for years it has not gained favour for measuring the quality of drinking or surface waters. The reason for this is that the ATP measured in the above industries is from the total microbial populations rather than from specific faecal indicator microorganisms used to measure the quality of water. This shortcoming has been overcome by the availability of magnetic beads coated with antibodies specific for the indicator bacteria used to measure water quality. The antibody captures the specific indicator bacterium and the captured cells are separated from the remainder of the sample with magnets. The separated cells are then assayed for ATP. The perceived need for more rapid methods for measuring water quality, especially in the areas of recreational waters and drinking water security, has established new interest in the use of luminescence. Measuring ATP has several advantages with regard to measuring water quality. This technology is relatively inexpensive, the results can be obtained in a very short time, the test is very sensitive and the technique can be used to measure analytes other than ATP. Furthermore, ATP can be used to estimate the number of viable cells in

a water sample. There is a paucity of research information on the use of the measurement of ATP for monitoring water quality. There are, in fact, no references to the use of ATP for measuring faecal indicators in marine waters or shellfish meats.

Lee and Deininger (2004) did show the use of this technology for measuring *E. coli* in fresh surface waters. Their method consisted of the following steps. Between 100 and 500 ml of water sample was first passed through a nylon pre-filter with a pore size of 20 μ to separate large particles from the sample. Following the pre-filter step, the sample was passed through a 0.45 μ filter. The retained cells were washed from the filter with phosphate buffered saline (PBS) containing Tween 20. The bacterial suspension was then mixed with anti-*E. coli* antibody adsorbed to the surface of magnetic beads. The solution was mixed for 15 minutes at 60 RPM. The *E. coli* captured by the beads were removed and concentrated from the buffer solution with a magnet applied to the side of the tube. The buffer solution was discarded and the captured cells were then washed twice in PBS. After washing, the retentate was suspended in 1 ml PBS and pipetted to a centrifuge tube. The magnet was applied again to separate the cells from the PBS, followed by the addition of 50 μl of somatic cell releasing agent. This step removed the non-bacterial ATP from the mixture. After further magnetic separation, the buffer was removed and the retentate was washed with PBS. After a fourth magnetic separation, the PBS was discarded and the ATP was extracted from the cells by a solution that dissolved the *E. coli* cell wall. This solution was transferred to a cuvette and 50 μl of luciferin/ luciferase solution was added for light development. The light emission was measured in relative light units (RLU) with a microluminometer.

This approach to measuring water quality shows some promise for *E. coli*. The detection limit is about 20 cfu/100 ml. The ATP measurement method results in an underestimate of the *E. coli* densities as measured by a membrane filter method. This ATP method was evaluated by a second group (Bushon *et al.* 2004) who compared it to a membrane filter method at three sites along a freshwater river. The correlation of the ATP method with the membrane filter method was reasonably good at two of the sites, but showed no relationship to the membrane filter method at the third sampling site. It was recognized that further research would be required to optimize the effectiveness of this method.

7.3 ENZYMATIC APPROACH

Specific enzymes in indicator bacteria have been used for many years to quantify the microbes associated with faeces. Enzymes, such as galactosidase, glucosidase and glucuronidase, are detected through the use of specific fluorogenic

or chromogenic substrates, such as 4-methylumbelliferyl-beta-D-galactoside, 4-methylumbelliferyl-beta-D-glucoside and indoxyl-beta-D-glucuronide. The substrates, which are colourless in the conjugated state, either fluoresce or present a colour after they have been hydrolyzed to form a fluorogenic or chromogenic compound and a sugar or an acid. In the culture approach, the substrates are incorporated into culture media for estimating the number of microbes in a water sample by the MPN or membrane filter procedures. These chromogens and fluorogens are used to specifically differentiate the target organisms from other bacteria that might grow on the selective media. These media generally require 24 hours or more for growth of colonies for an estimate of their density.

Enzymatic methods followed two approaches for quantifying coliforms and faecal coliforms. The first approach for measuring faecal coliforms is to pipette a fluorogenic substrate directly into a mixture of water sample and buffer. The total volume of the solution is 12 ml. The mixture is incubated for one hour at 44.5°C. After the incubation period the medium is cooled very rapidly to stop the enzyme reaction and the solution is adjusted to pH 10 to optimize the fluorescent intensity. A fluorescent calibration curve is produced relating standard concentrations of the fluorogen to fluorescent intensity. The fluorescent intensity of an unknown sample is compared to the standard curve to estimate the number of cells in the sample.

A second approach involves the incubation of a membrane filter in a flask with 12 ml of buffer, fluorogen and a surfactant. The water sample (100 ml) is passed through the membrane filter before the membrane is placed in a 200 ml flask. The mixture is incubated in a water bath at 44°C. Every 5 minutes for 30 minutes a 2 ml aliquot is removed and placed in a cuvette containing sodium hydroxide. The fluorescent intensity is measured with a spectrofluorometer and is expressed as the amount of fluorescence liberated per minute for 100 ml of sample. The time interval at which the fluorescence is first detected is related to the number of bacteria in the sample.

Davies and Apte (1999) examined 254 water samples for faecal coliforms. The enzymatic test measured the hydrolysis of 4-methyl-umbellferyl-beta-D-galactoside. The results showed a linear increase between fluorescent intensity and colony forming units (cfu) above 300 cfus. Below 300 faecal coliforms there was no relationship between fluorescent intensity and cfus. It was suggested that this enzymatic test could be used in the presence/absence mode. Lebaron et al. (2005) examined the rate of hydrolysis of a fluorogenic substrate, 4-methylumbelliferyl-beta-D-glucuronide, to detect the presence at E. coli in seawater. Twenty-six beach water samples were assayed using the multi-well MPN cultural procedure and a method using enzymatic hydrolysis of a substrate

over time to measure the *E. coli* density in the water sample. The results for both assays were compared and the findings showed that the results were similar, however the enzymatic results were somewhat higher than those from the cultural procedure. The authors attributed this to multiple *E. coli* cells that may have attached to particles and were counted as one cell in the MPN method. They also found the limit of sensitivity of the enzymatic method was about 5 cells/per 100 ml.

These methods may provide more rapid measurements of water quality. The enzymatic endpoint approach described by Davis and Apte (1999) would have limited application to shellfish harvesting waters because of the limit of sensitivity, which is rather high. The limit of sensitivity of the enzymatic hydrolysis of substrate approach (LeBaron 2004), on the other hand, is low, well within the range of shellfish harvesting water coliform limits.

7.4 CONCLUSIONS

The methods described above have the potential to measure the quality of both types of samples in a timely manner. Situations where rapid monitoring methods might be used include shellfish harvesting waters contaminated by an accidental sewage spill when it is important to know as soon as possible that the waters have returned to ambient conditions. There may also be instances where it is critical to know if harvested shellfish are contaminated and this information must be obtained in a timely manner. The methods discussed here are reported to furnish results in approximately one (Davies and Apte 1999; Lee and Deininger 2004; LeBaron *et al.* 2005) to four hours (Haugland *et al.* 2005).

It is frequently important to know if the pathogens being measured are viable and therefore able to cause infections. The available molecular methods are not able to identify and quantify viable microbes. Quantitative PCR measures both viable and non-viable bacteria and, therefore, this technique gives results that are greater than those obtained with cultural methods. Chemiluminescence and enzymatic methods, on the other hand, do measure viable microbes. Therefore, the estimates of bacterial densities in water samples by those methods are more likely to be comparable to those obtained with cultural methods.

The sensitivity of these methods is very good. All of these methods should, in theory, be able to measure one cell. In practice, samples for the qPCR technique and the chemiluminescence technique must be concentrated using membrane filtration. The former method requires that 100 ml of the sample is filtered for the assay and the latter method requires filtration of 100–500 ml of sample for the assay. The enzymatic test described by Davis and Apte (1999) had a limit of sensitivity of about 300 cells. They did not discuss the use of a concentration

step as a means of increasing the sensitivity of the test. The procedure that measured the rate of hydrolysis of a substrate (LeBaron *et al.* 2005) had a limit of sensitivity of five microbial cells using a 100 ml water sample, which is well within the range needed to measure currently-used indicator bacteria without further concentration of the sample.

The specificity of the new technologies for measuring microbes associated with faeces is frequently much better than that observed with cultural methods. This is especially true for molecular methods that detect unique portions of the genome of the indicator bacterium or pathogen. Methods that use antibody capture may also be highly specific, depending on the quality of the antibodies attached to the magnetic beads. Enzymatic methods may at times show some non-specificity with regard to target analytes. If non-target microorganisms with enzyme systems similar to the microorganisms of interest are contained in the water sample, it is possible that they might hydrolyze the substrate and cause a false positive result to occur. This situation would probably not occur more frequently than it would using a cultural approach.

There are several excellent reviews in the literature which address new methods and techniques for detecting and quantifying microbes in water and other media. Two of the reviews (Sidorowicz and Whitmore 1995; Rompre 2002) specifically address the rapid monitoring of coliforms in drinking water. The two papers describe the better-known of the rapid technologies such as flow cytometry, in situ hybridization, the PCR and enzyme-based approaches, as well as their advantages and disadvantages. A review of biosensors describes some of the more esoteric methods and techniques such as optical transducers, bioluminescence sensors, piezoelectric biosensors systems and electrical impedance biosensors. Other biosensors based on fluorescence labelled antibodies or electrochemical immunodetection and flow immunosensors are also discussed (Ivnitski *et al.* 1999). Although many of the detection and enumeration systems described in this review are not available to measure microbes in water and food, they do provide heuristic examples of some of the technologies that may become available in the future. Mandrell and Wachtel (1999) reviewed novel detection techniques for human pathogens that contaminate poultry. They addressed mainly the use of immunomagnetic separation and molecular techniques for a couple of the more common pathogens found in poultry, such as a *Salmonella* and *Campylobacter*, two bacterial pathogens that are sometimes associated with shellfish.

The rapid advances being made in the development of new methods and technologies for detecting and quantifying microbes in water and food, coupled with the great interest in maintaining the quality of shellfish harvesting waters, will undoubtedly lead to greater use of high technology, rapid methods in

the future. The many advantages of these new and emerging technologies are already evident and they provide a sound basis for transition from the methods of the last century to new means for protecting public health through better monitoring of the quality of our foods and water.

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8

Sanitary profiling of shellfish harvesting areas

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Sanitary surveys, or profiling, is an appraisal tool designed to provide a human health risk assessment for specific shellfish harvesting areas. It has evolved from the hazard analysis and critical control point (HACCP) concepts which first developed in the food processing industries to ensure appropriate 'sanitary' control of production steps which could involve a risk of contamination. The rationale for this approach is that, where the principal contamination risk derives from microbial pathogens, end-product testing of 'fresh' food may be impractical due to the timing of information delivery on pathogen concentration and the proportion of the product needing analysis. In such situations, HACCP is used to maintain product quality in food production and processing facilities. It is, therefore, a relatively small step to see this process transferred from the

factory back up the production chain to the environment in which fresh product is grown. Indeed, where a premium product is consumed uncooked shortly after harvest, this may be the only practical means of maintaining public health protection. Very similar concepts have been applied in recreational and drinking water management (WHO 2003; 2004) and these are outlined in chapter 15. The difference between sanitary profiling and HACCP is that the former remains a means of problem scoping whilst the latter has evolved into a management tool designed to maintain real time sanitary security where public health protection is the central management responsibility.

Thus, sanitary profiling should be considered one tool in a management armoury (Todd and Campbell 2002) which should encompass:

- the sanitary surveillance and evaluation of shellfish growing water catchments;
- routine sampling of growing waters and the shellfish for bacteriological and chemical parameters;
- the analyses of results and classification of growing waters.

8.1 INTERNATIONAL APPLICATION

Sanitary surveys are an obligatory component of the assessment of the faecal pollution status of harvesting areas in both the United States National Shellfish Sanitation Programme (NSSP) (US FDA 2008) and the European Union (EU) Food Hygiene Regulations (European Communities 2004). For the United States programme, this means that they are required for areas within the United States involved in interstate trade and also for areas in countries with a Memorandum of Understanding with the US Food and Drug Administration (FDA) and from which product is exported to the United States. For the EU programme, sanitary surveys are required for all harvesting areas classified after 1 January 2006 in EU Member States, countries of the European Free Trade Association (EFTA), and also in other countries approved for exporting bivalve molluscs to the EU.

The United States NSSP states that:

> The sanitary survey is the written evaluation report of all environmental factors, including actual and potential pollution sources, which have a bearing on water quality in a shellfish growing area.

The sanitary survey shall include the data and results of:

- a shoreline survey;
- a survey of the bacteriological quality of the water;

- an evaluation of the effect of any meteorological, hydrodynamic, and geographic characteristics on the growing area;
- an analysis of the data from the shoreline survey, the bacteriological and the hydrodynamic, meteorological and geographic evaluations; and
- a determination of the appropriate growing area classification.

An annual review is undertaken to ensure that information is up to date and a more extensive re-evaluation is undertaken every three years. A completely new sanitary survey has to be undertaken every 12 years. The outcome of the sanitary survey is a report containing the information from the survey, the recommended classification for the area and the boundaries of the classified area. The NSSP guide gives more detail on the application of sanitary surveys. This is supplemented by courses run by the FDA. A detailed account of the United States approach has been published by Garreis (1994).

In the EU, the sanitary survey requirements are specified in the Food Hygiene Regulations (Anon 2004). These state that, if the competent authority decides in principle to classify a production or relaying area, it must:

- make an inventory of the sources of pollution of human or animal origin likely to be a source of contamination for the production area;
- examine the quantities of organic pollutants which are released during the different periods of the year, according to the seasonal variations of both human and animal populations in the catchment area, rainfall readings, wastewater treatment and other similar factors;
- determine the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area.

These outline requirements are expanded into recommendations given in a Good Practice Guide for the Microbiological Monitoring of Shellfish Harvesting Areas (Anon 2007). This proposes that sanitary surveys consist of the following elements:

(1) Desk study – compiling information from existing sources on the fishery, potential sources of faecal pollution (human and animal) and meteorology.
(2) Shoreline survey – to confirm the findings of the desk study and to determine other sources not identified during the desk study.
(3) Bathymetry and hydrodynamics – to determine whether, and to what extent, any potential sources of faecal pollution impact on the shellfishery.

(4) Bacteriological survey – a short-term sampling programme of seawater and/or shellfish undertaken in support of the sanitary survey and prior to ongoing routine monitoring of the harvesting area.

The outcome of the sanitary survey is a report that recommends the sampling plan for ongoing monitoring for classification, together with the boundaries of the area that will be classified on the basis of that monitoring.

An important difference between the United States and EU approaches is that the former includes a review of the classification status of the area while the latter primarily informs the sampling plan on which the monitoring required by that classification is based. In the United States approach, the hydrodynamic assessment often includes dye tracing studies to inform dilution estimates and the determination of the extent of closure zones around outfalls. The latter is not considered in the EU Regulation. The United States approach to classification also includes the possibility of conditional classifications (see chapter 6 and chapter 9) and a significant component of the sanitary survey may be devoted to determine whether contamination is significantly worse under defined and predictable conditions and, if so, the criteria that should determine when the closure (or depuration, if appropriate) should be initiated and subsequently lifted.

8.2 THE CATCHMENT CONTEXT

Shellfish are grown in nearshore waters subject to terrestrial fluxes of faecal indicator bacteria from: treated and untreated human sewage discharges (Table 8.1); animal wastes from farms, pets and working draft animals; as well as avian and mammalian wildlife (Bidwell and Kelly 1950). These, mainly terrestrial, sources produce highly episodic input fluxes to the shellfish harvesting waters driven by rainfall events which generate the transport energy to deliver the faecal indicator shellfish compliance parameters to the nearshore zone (Wilkinson *et al.* 2006). Some inputs may be direct to the marine environment from aquatic mammals, birds and boats.

Once in the nearshore zone, hydrodynamic processes, driven by tide and wind, dominate the transport from the input locations to the harvesting waters. There can also be transport of faecal indicators from other marine environments which might be relatively distant where the survival of these gut bacteria is prolonged. Such conditions are favoured by high turbidity (which provides attachment sites and protection from ultra violet (UV) irradiance), low light intensity (which reduce the bactericidal effects of UV irradiance) and rapid current speeds (which reduce the time available for bactericidal processes to have an effect) (Sinton *et al.* 2002; Kay *et al.* 2005a).

Table 8.1 Sewage treatment types sampled in Kay *et al.* (2008c)

Level of treatment[a]	Specific effluent types[a]
Untreated sewage (69)	Crude sewage discharges (16)
	Storm sewage overflows[b] (53)
Primary treatment (12)	Primary settled sewage effluent (7)
	Stored settled sewage effluent (2)
	Settled septic tank effluent (3)
Secondary treatment (67)	Trickling filter effluent (38)
	Activated sludge effluent[c] (17)
	Oxidation ditch effluent (3)
	Trickling/sand filter effluent (1)
	Rotating biological contactor effluent (8)
Tertiary treatment (14)	Reedbed/grass plot effluent (6)
	Ultraviolet-disinfected effluent (8)

[a] Figures in brackets indicate number of different treatment plants sampled (numbers of valid enumerations (n) are shown in Table 8.2).
[b] Comprise treatment plant inlet overflows, stormwater retention tank overflows and combined sewer overflows; high-flow data only.
[c] Includes deep-shaft activated sludge effluent at one site.

Clearly, effective improvement of poor water quality in nearshore shellfish harvesting waters depends on a good knowledge of the relative importance and impact of the various sources of bacteria from catchment and other sources. This field of 'quantitative microbial source apportionment' (QMSA) is addressed in chapter 15 which explains its development in the management of recreational waters. Sanitary survey of shellfish harvesting waters seeks to acquire the information for a largely 'qualitative' assessment of likely source importance. It can inform remediation strategies and ensure appropriate focus on key and likely sources in the absence of detailed quantitative information. However, where the fishery is of economic or societal importance and significant resource commitment to remediation of identified pollution is intended, then it would be prudent to validate any qualitative sanitary survey with empirical data from a properly conducted QMSA investigation (see chapter 15).

8.3 ASSESSMENT OF INPUT FLUXES

The most basic information needed by any agency seeking to implement a sanitary profile of a shellfish harvesting area is the likely microbial flux from a range of potential catchment and nearshore sources. Surprisingly, peer reviewed data describing even the common faecal indicators in a range of effluents and faeces is sparse. Similarly, information on likely fluxes of these parameters from agricultural diffuse pollution is almost absent. This contrasts with, for example,

parameters measured in sewage effluents indicative of impacts on riverine salmonid and cyprinid fisheries such as BOD and ammoniacal nitrogen, or parameters indicative of eutrophication such as phosphorus for riverine discharges or nitrogen for marine outfalls. The reason for this poor data availability is the historical absence of the faecal indicators in regulatory consents for riverine or, indeed, marine discharges.

8.3.1 Human sewage discharges

Human sewage effluents from urban populations will always be the faecal indicator flux of principal concern to shellfish growers because these fluxes have the highest probability of containing viral pathogens such as human noroviruses which have been proven to cause shellfish associated illness (Lee and Kay 2006). Empirical data describing 'infective' norovirus concentrations in a range of sewage effluents is not available. However, some peer reviewed information on the faecal indicator compliance parameters is now in the public domain (Kay *et al.* 2008c). These inputs to nearshore waters are discharged via pipes and outfalls and are commonly termed 'point sources' of pollution.

These data were acquired in a series of empirical investigations outlined in Kay *et al.* (2008c). Most of these systems were treating raw effluent delivered to the sewage treatment plant through, so called, 'combined' sewerage systems. These systems have the capacity to accommodate urban drainage after periods of rainfall when the volume of untreated affluent increases to perhaps three to five times the 'dry weather flow'. The treatment system installed will be designed to take this increased flow which is termed 'flow to full treatment'. If the flow increases beyond this level the excess is either stored in tank systems within the sewerage network infrastructure and/or at the sewage treatment plant, or, if this infrastructure capacity is exceeded, the raw sewage will spill to a river or coastal waters via 'combined sewer overflows' (CSO) from the sewerage network or from a 'storm tank overflow' (STO) from the storage tanks. These are labelled as 'storm sewage overflows' in Table 8.2 and a quality value for these discharges are only listed for high flow conditions when they commonly operate. Both 'base flow' (dry weather) and 'high flow' (wet weather) values are reported in Table 8.2. These are values for treated sewage effluents which reflect the altered plant retention times following increased flows during rainfall events. Whilst these data come from a restricted geographical area (the United Kingdom), the treatment types are relatively widespread in temperate developed nations. However, significant data gaps remain in the available empirical data resource describing effluent quality produced by tropical and semi-tropical treatment systems and particularly in developing nations.

Table 8.2 Summary of faecal indicator organism concentrations (cfu 100 ml⁻¹) for different treatment levels and individual types of sewage-related effluents under different flow conditions: geometric means (GMs), 95% confidence intervals (CIs)[a]; and results of t-tests comparing base- and high-flow GMs for each group and type[b]; and (in footnote) results of t-tests comparing GMs for the two untreated discharge types and the two tertiary-treated effluent types

Indicator organism / Treatment levels and specific types	Base flow conditions				High flow conditions			
	n[c]	Geometric mean	Lower 95% CI	Upper 95% CI	n[c]	Geometric mean	Lower 95% CI	Upper 95% CI
Faecal coliforms								
Untreated								
Crude sewage discharges[d]	**252**	**1.7×10^7 *(+)**	**1.4×10^7**	**2.0×10^7**	**282**	**2.8×10^6 *(−)**	**2.3×10^6**	**3.2×10^6**
Storm sewage overflows[d]	252	1.7×10^7 *(+)	1.4×10^7	2.0×10^7	79	3.5×10^6 *(−)	2.6×10^6	4.7×10^6
					203	2.5×10^6	2.0×10^6	2.9×10^6
Primary	**127**	**1.0×10^7 *(+)**	**8.4×10^6**	**1.3×10^7**	**14**	**4.6×10^6 *(−)**	**2.1×10^6**	**1.0×10^7**
Primary settled sewage	60	1.8×10^7	1.4×10^7	2.1×10^7	8	5.7×10^6	–	–
Stored settled sewage	25	5.6×10^6	3.2×10^6	9.7×10^6	1	8.0×10^5	–	–
Settled septic tank	42	7.2×10^6	4.4×10^6	1.1×10^7	5	4.8×10^6	–	–
Secondary	**864**	**3.3×10^5 *(−)**	**2.9×10^5**	**3.7×10^5**	**184**	**5.0×10^5 *(+)**	**3.7×10^5**	**6.8×10^5**
Trickling filter	477	4.3×10^5	3.6×10^5	5.0×10^5	76	5.5×10^5	3.8×10^5	8.0×10^5
Activated sludge	261	2.8×10^5 *(−)	2.2×10^5	3.5×10^5	93	5.1×10^5 *(+)	3.1×10^5	8.5×10^5
Oxidation ditch	35	2.0×10^5	1.1×10^5	3.7×10^5	5	5.6×10^5	–	–
Trickling/sand filter	11	2.1×10^5	9.0×10^4	6.0×10^5	8	1.3×10^5	–	–
Rotating biological contactor	80	1.6×10^5	1.1×10^5	2.3×10^5	2	6.7×10^5	–	–
Tertiary	**179**	**1.3×10^3**	**7.5×10^2**	**2.2×10^3**	**8**	**9.1×10^2**	–	–
Reedbed/grass plot[e]	71	1.3×10^4	5.4×10^3	3.4×10^4	2	1.5×10^4	–	–
Ultraviolet disinfection[e]	108	2.8×10^2	1.7×10^2	4.4×10^2	6	3.6×10^2	–	–

Notes: [a] CIs only reported where $n \geq 10$; [b] t-tests comparing low- and high-flow GM concentrations only undertaken where $n \geq 10$ for both sets of samples; only statistically significant ($p < 0.05$) differences between base- and high-flow GM concentrations are reported: indicated by *, with the higher GM being identified as *(+) and the lower value by *(−); [c] n indicates number of valid enumerations, which in some cases may be less than the actual number of samples; [d] t-tests comparing the GM concentrations between the two untreated discharge types show high-flow GM concentrations to be significantly higher in crude sewage discharges than storm sewage discharges ($p < 0.001$); [e] t-tests comparing the GM concentrations between the two tertiary-treatment effluent types show GM TC, FC and EN concentrations to be significantly higher ($p < 0.001$) in reedbed/grass plot effluents than effluents from UV disinfection for base-flow conditions (there are too few high-flow samples for these tertiary effluents for meaningful comparisons to be made for high-flow GM concentrations).
Source: Kay *et al.* 2008c

The utility of the data in Table 8.2 to the manager seeking to construct a sanitary profile for a shellfish harvesting water are that a total flux can be estimated using the concentration for each point source of treated effluent in a catchment and the flow of effluent discharged. In many rural environments served by small and possibly old sewage treatment plants, there may be no direct measurement of the sewage flow through the treatment plant. In such situations, the population served may provide a reasonable estimate of the likely sewage volume generated. In the United Kingdom, an estimate of 160–185 litres per person per day is often assumed. However, it should be appreciated that aging sewerage systems can suffer ingress of groundwater and lose effluent through leakage, thus there may be wide system-specific variability in such flow estimates.

If the manager wishes to split the effluent flow into a base flow and high flow component, then flow information for a specific plant is required. This is an important consideration because the principal period of shellfish contamination is often following rainfall when other fluxes also peak. Thus, characterisation of the treated sewage point source flux during these periods is crucial to an accurate assessment of high flow flux and, importantly, accurate estimates of the likely beneficial effects of the significant investments needed to reduce faecal indicator fluxes from treated sewage effluents.

Perhaps the most contentious and least understood point source discharges are the CSOs and STOs. These discharge raw (untreated) sewage diluted by rainfall which may be considered by the lay public as less aesthetically acceptable than a 'treated' sewage effluent discharged from a sewage treatment works. Very few CSOs or STOs in rural areas would have flow monitoring fitted to the discharge location. They are often regulated by the definition of an allowed spill frequency per year (Lee *et al.* 2002) and, hence, their contribution to the faecal indicator flux for a specific location under a defined rainfall sequence is often difficult to quantify. In QMSA studies of recreational waters they have been seen to contribute a wide range of inputs for different catchments. They may indeed be the largest single element of the flux in urban catchments with aging sewerage infrastructure (Kay *et al.* 2005c; Wither *et al.* 2005; Stapleton *et al.* 2008), but in rural environments the CSO and STO components may be relatively trivial (Crowther *et al.* 2002; 2003).

8.3.2 Diffuse catchment sources of faecal indicator organisms

Faecal indicator fluxes from the catchment land surface are commonly termed 'diffuse source pollution'. Modelling diffuse source pollution is a well established science, particularly for the nutrient parameters (Haygarth *et al.* 2005; Heathwaite *et al.* 2005a; 2005b). Such models often employ the concept

of 'export coefficients' for the pollutant of interest. This can be expressed as the weight of pollutant (such as kg) lost from each unit area (such as km^2) per unit time (such as year). These export coefficients can be refined by assessment of seasonal patterns which may, for example, dictate reporting of a summer and a winter coefficient and/or flow conditions in the exporting streams which may dictate a low and high flow export coefficient. Considerable efforts in the developed nations has been devoted to the calculation and reporting of export coefficients for nutrients and priority substances needed to provide the evidence base for implementation of the EU Water Framework Directive and United States Clean Water Act described in chapter 15. However there are no globally available export coefficients for different land use assemblages and climatic conditions reported to date.

Faecal indicator concentrations in streams and export coefficients from sub-catchments for a range of United Kingdom studies under temperate northern European land use have been recently reported (Kay *et al.* 2008b). Table 8.3 lists the study catchments, the land use data sources accessed and the sample numbers analysed. Table 8.4 provides characteristic base flow and high flow faecal indicator concentrations in catchment streams generated by diffuse catchment sources of faecal indictors. Table 8.5 provides export coefficients (log_{10} cfu km^{-2} hr^{-1}) for base flow and high flow conditions under different land use assemblages commonly encountered in rural north-temperate Europe.

Given the emerging role of faecal indicators in water quality 'impairments' identified under the requirements of the United States Clean Water Act (Kay *et al.* 2008a), it is likely that the demand for export coefficient information describing the faecal indicator flux from common land use types will grow dramatically. The early lessons of the United Kingdom work suggest that, in comparable climatic zones, the export is: (i) highly episodic and driven by high flow events during which bacteria are transported to stream channels and then out of the catchment system (Tyrrel and Quinton 2003; Oliver *et al.* 2005a; 2005b); and (ii) that the flux is highly seasonal with a peak in summer when livestock are commonly grazed in fields before being housed in the winter (Rodgers *et al.* 2003; Kay *et al.* 2005b; 2007a; 2007b). Consideration of these patterns has not, to date, figured highly in large catchment modelling (Tong and Chen 2002).

The data in Tables 8.4 and 8.5 can be used to generate flux information by combining available river flow data with the concentration data in Table 8.4 and using the export coefficient data in Table 8.5 directly to estimate flux in the absence of reliable continuous flow records. However, this assumes similar climatic characteristics and land use patterns to temperate northern Europe and this assumption needs careful consideration and testing.

Table 8.3 Catchments and data sources for faecal indicator export coefficients for faecal indicators

Catchment	Year	Number of subcatchments[a]	Land use data[b]
England			
River Leven/Crake	2005	30	CEH2000/OS
Holland Brook	1998	14	Field mapping/OS
River Ribble	2002	40	ITE1990/OS
Staithes Beck	1995	4	Field mapping/OS
Windermere (lake) inputs	1999	25	CEH 2000/OS
Scotland			
Brighouse Bay inputs	2004	2 (2)	Estimated
Ettrick Bay inputs	2004	3 (2)	SE
River Irvine/Garnock	1998	30	Field mapping + MLCMS
Killoch Burn[c]	2004	4 (3)	SE
River Nairn	2004	1	SE
Sandyhills	2004	4 (4)	SE
Troon coastal inputs	2000	6	Estimated
Wales			
Afon[d] Nyfer	1996	2	Field mapping/OS
Afon[d] Ogwr	1997	18	Field mapping/OS
Afon[d] Rheidol/Ystwyth	1999	22	Field mapping/OS

Notes: [a] Numbers of subcatchments used in summer/winter comparisons are shown in parentheses; [b] Land use data sources: Estimated = estimates for the two key land use types: built-up land (from OS 1:50 000 maps) and improved pasture (from field reconnaissance); Field mapping/MLCMS = land use mapping during study period of part of the catchment, supplemented by the 1988 Macaulay Land Cover Map of Scotland, calibrated through field mapping; Field mapping/OS = land use mapping during study period, supplemented by Ordnance Survey 1:50 000 digital map information for built-up land and woodland; ITE1990/OS = Institute of Terrestrial Ecology Land Cover for 1990, calibrated using ground truth data from the five study areas in England and Wales where field mapping was undertaken, and supplemented by Ordnance Survey 1:50 000 digital map information for built-up land and woodland; CEH2000/OS = Centre for Ecology and Hydrology Land Cover Map for 2000, supplemented by Ordnance Survey 1:50 000 digital map information for built-up land and woodland; and SE = Land use data generated by Scottish Executive; [c] Killoch Burn is located within the headwaters of the River Irvine/Garnock catchment; [d] 'Afon' (Welsh) = 'River'.
Source: Kay *et al.,* 2008b

8.3.3 Managing the complexity

The implications of these observations for sanitary profiling of catchments are that: (i) both high and low flow should be estimated and reported separately, the total flux being derived from the summation of the high and low flow elements; and (ii) seasonal patterns should be considered and, where required by either the

Table 8.4 Geometric mean (GM) and 95% confidence intervals (CIs) of the GM faecal indicator organism (FIO) concentrations (cfu 100 ml^{-1}) under base- and high-flow conditions at the 205 sampling points and for various subsets, and results of paired t-tests to establish whether there are significant elevations at high flow compared with base flow

Faecal indicator organisms		Base flow			High flow		
Subcatchment land use	n	Geometric mean	Lower 95% CI	Upper 95% CI	Geometric mean[a]	Lower 95% CI	Upper 95% CI
Total coliforms							
All subcatchments	205	5.8×10^3	4.5×10^3	7.4×10^3	7.3×10^{4}**	5.9×10^4	9.1×10^4
Degree of urbanisation[b]							
Urban	20	3.0×10^4	1.4×10^4	6.4×10^4	3.2×10^{5}**	1.7×10^5	5.9×10^5
Semi-urban	60	1.6×10^4	1.1×10^4	2.2×10^4	1.4×10^{5}**	1.0×10^5	2.0×10^5
Rural	125	2.8×10^3	2.1×10^3	3.7×10^3	4.2×10^{4}**	3.2×10^4	5.4×10^4
Rural subcatchments with different dominant land uses							
≥75% Improved pasture	15	6.6×10^3	3.7×10^3	1.2×10^4	1.3×10^{5}**	1.0×10^5	1.7×10^5
≥75% Rough grazing	13	1.0×10^3	4.8×10^2	2.1×10^3	1.8×10^{4}**	1.1×10^4	3.1×10^4
≥75% Woodland	6	5.8×10^2	2.2×10^2	1.5×10^3	6.3×10^{3}*	4.0×10^3	9.9×10^3
Degree of urbanisation[b]							
Urban	20	9.7×10^3	4.6×10^3	2.0×10^4	1.0×10^{5}**	5.3×10^4	2.0×10^5
Semi-urban	60	4.4×10^3	3.2×10^3	6.1×10^3	4.5×10^{4}**	3.2×10^4	6.3×10^4
Rural (<2.5% built-up land)	125	8.7×10^2	6.3×10^2	1.2×10^3	1.8×10^{4}**	1.3×10^4	2.3×10^4
Rural subcatchments with different dominant land uses							
≥75% Improved pasture	15	1.9×10^3	1.1×10^3	3.2×10^3	5.7×10^{4}**	4.1×10^4	7.9×10^4
≥75% Rough grazing	13	3.6×10^2	1.6×10^2	7.8×10^2	8.6×10^{3}**	5.0×10^3	1.5×10^4
≥75% Woodland	6	3.7×10	1.2×10	1.2×10^2	1.5×10^{3}**	6.3×10^2	3.4×10^3

(*continued*)

Table 8.4 *Continued*

Faecal indicator organisms Subcatchment land use	n	Base flow			High flow		
		Geometric mean	Lower 95% CI	Upper 95% CI	Geometric mean[a]	Lower 95% CI	Upper 95% CI
Enterococci							
All subcatchments	205	2.7×10^2	2.2×10^2	3.3×10^2	5.5×10^{3}**	4.4×10^3	6.8×10^3
Degree of urbanisation[b]							
Urban	20	1.4×10^3	9.1×10^2	2.1×10^3	2.1×10^{4}**	1.3×10^4	3.3×10^4
Semi-urban	60	5.5×10^2	4.1×10^2	7.3×10^2	1.0×10^{4}**	7.6×10^3	1.4×10^4
Rural (<2.5% built-up land)	125	1.5×10^2	1.1×10^2	1.9×10^2	3.3×10^{3}**	2.4×10^3	4.3×103
Rural subcatchments with different dominant land uses							
≥75% Improved pasture	15	2.2×10^2	1.4×10^2	3.5×10^2	1.0×10^{4}**	7.9×10^3	1.4×10^4
≥75% Rough grazing	13	4.7×10	1.7×10	1.3×10^2	1.2×10^{3}**	5.8×10^2	2.7×10^3
≥75% Woodland	6	1.6×10	7.4	3.5×10	1.7×10^{2}**	5.5×10	5.2×10^2

Notes: [a] Significant elevations in concentrations at high flow are indicated: ** $p < 0.001$, * $p < 0.05$; [b] Degree of urbanisation, categorised according to percentage built-up land: 'Urban' (≥10.0%), 'Semi-urban' (2.5–9.9%) and 'Rural' (<2.5%).
Source: Kay *et al.* 2008b

Table 8.5 Summary of geometric mean faecal indicator organism (FIO) export coefficients (cfu km^{-2} hr^{-1}) under base- and high-flow conditions at the 205 sampling points and for various subsets, and results of paired 1-tailed t-tests to establish whether there are significant elevations at high flow compared with base flow

Faecal indicator organisms		Base flow			High flow		
Subcatchment land use	n	Geometric mean	Lower 95% CI	Upper 95% CI	Geometric mean[a]	Lower 95% CI	Upper 95% CI
Total coliforms							
All subcatchments	205	1.8×10^9	1.4×10^9	2.4×10^9	9.5×10^{10}**	7.2×10^{10}	1.2×10^{11}
Degree of urbanisation[b]							
Urban	20	8.5×10^9	3.3×10^9	2.2×10^{10}	4.1×10^{11}**	1.6×10^{11}	1.1×10^{12}
Semi-urban	60	4.2×10^9	2.6×10^9	6.7×10^9	1.5×10^{11}**	8.3×10^{10}	2.7×10^{11}
Rural	125	9.3×10^8	6.9×10^8	1.3×10^9	6.1×10^{10}**	4.6×10^{10}	8.0×10^{10}
Rural subcatchments with different dominant land uses							
≥75% Improved pasture	15	2.9×10^9	1.4×10^9	6.0×10^9	2.8×10^{11}**	1.6×10^{11}	4.9×10^{11}
≥75% Rough grazing	13	7.1×10^8	3.5×10^8	1.4×10^9	5.3×10^{10}**	2.6×10^{10}	1.1×10^{11}
≥75% Woodland	6	3.1×10^8	5.7×10^7	1.6×10^9	1.4×10^{10}**	6.0×10^9	3.4×10^{10}
Faecal coliforms							
All subcatchments	205	5.5×10^8	4.1×10^8	7.2×10^8	3.6×10^{10}**	2.7×10^{10}	4.8×10^{10}
Degree of urbanisation[b]							
Urban	20	2.8×10^9	1.1×10^9	7.2×10^9	1.3×10^{11}**	4.8×10^{10}	3.6×10^{11}
Semi-urban	60	1.2×10^9	7.4×10^8	1.9×10^9	4.6×10^{10}**	2.5×10^{10}	8.6×10^{10}
Rural (<2.5% built-up land)	125	2.9×10^8	2.1×10^8	4.0×10^8	2.6×10^{10}**	1.9×10^{10}	3.5×10^{10}
Rural subcatchments with different dominant land uses							
≥75% Improved pasture	15	8.3×10^8	4.3×10^8	1.6×10^9	1.2×10^{11}**	6.5×10^{10}	2.2×10^{11}
≥75% Rough grazing	13	2.5×10^8	1.1×10^8	5.7×10^8	2.5×10^{10}**	1.1×10^{10}	5.5×10^{10}
≥75% Woodland	6	2.0×10^7	4.7×10^6	8.2×10^7	3.3×10^9**	1.3×10^9	8.8×10^9

(continued)

Table 8.5 *Continued*

Faecal indicator organisms Subcatchment land use	n	Base flow			High flow		
		Geometric mean	Lower 95% CI	Upper 95% CI	Geometric mean[a]	Lower 95% CI	Upper 95% CI
Enterococci							
All subcatchments	205	8.3×10^7	6.6×10^7	1.1×10^8	7.1×10^{9}**	5.5×10^9	9.3×10^9
Degree of urbanisation[b]							
Urban	20	4.0×10^8	2.1×10^8	7.6×10^8	2.7×10^{10}**	1.1×10^{10}	6.2×10^{10}
Semi-urban	60	1.5×10^8	9.8×10^7	2.2×10^8	1.1×10^{10}**	6.1×10^9	1.9×10^{10}
Rural (<2.5% built-up land)	125	4.9×10^7	3.7×10^7	6.5×10^7	4.7×10^{9}**	3.5×10^9	6.3×10^9
Rural subcatchments with different dominant land uses							
≥75% Improved pasture	15	9.6×10^7	5.2×10^7	1.8×10^8	2.2×10^{10}**	1.3×10^{10}	3.8×10^{10}
≥75% Rough grazing	13	3.3×10^7	1.2×10^7	9.0×10^7	3.6×10^{9}**	1.3×10^9	9.7×10^9
≥75% Woodland	6	8.5×10^6	3.8×10^6	1.9×10^7	3.8×10^{8}**	1.3×10^8	1.1×10^9

[a] Significant elevations in export coefficients at high flow are indicated: ** $p < 0.001$
[b] Degree of urbanisation, categorised according to percentage built-up land: 'Urban' (≥10.0%), 'Semi-urban' (2.5–9.9%) and 'Rural' (<2.5%).
Source: Kay *et al.* 2008b

catchment characteristics and/or management requirements of the harvesting waters, reported separately. The principal reason for this apparently complex assessment framework is the potential need to inform appropriate remediation strategies which should target the principal flow conditions and seasonal periods associated with impaired quality of harvesting waters and shellfish flesh quality. Combined data describing, for example, annual export coefficients, may mask the importance of rainfall driven episodic events when quantification of the balance between point and diffuse faecal indicator fluxes may be a key piece of management information in allocating resources to competing remediation options designed to either disinfect treated sewage effluents and/or attenuate diffuse catchment fluxes of faecal indicator bacteria.

A further consideration which should be recognised is the different risk implications of the various faecal indicator sources in any catchment system. As noted above, the principal source of human enteric viruses, such as noroviruses, will be human sewage and associated treated effluents generally discharged as 'point sources'. The agricultural diffuse sources of faecal indicators are less likely to be associated with human pathogenic viruses, however, they will almost certainly contain zoonotic pathogens such as *Cryptosporidium* spp. and pathogenic *E. coli* (Stelma and McCabe 1992). Thus, the animal-derived catchment diffuse source pollution cannot be ignored as a disease risk but there may be a good rationale for an initial focus on the human sewage-derived fluxes of faecal indicators. However, in pure 'compliance' terms it is the faecal indicators which represent the legally required quality standard and the origin (animal or human) of such faecal indicators is irrelevant. Thus, animal-derived faecal indicators found in shellfish water or flesh can still cause a water to fail even though they may not index a high risk of human pathogenic virus presence. For this reason, it is important to be clear on the rationale for the sanitary profile which may be: (i) human health risk assessment; (ii) to underpin a strategy for compliance with standards in force; or (iii) a combination of these management drivers.

8.4 CONTENTS OF A SANITARY PROFILE

Many individual case studies of shellfish sanitation have been reported in the literature, most of which comprise at least some elements of a sanitary profile (Clem 1971; 1974; Mac Millan 1973; Sato *et al.* 1992; Busse 1998; Leonard 2001; Levesque *et al.* 2006; Ogawa 2006). There are recommended formats for reports under both the NSSP and EU systems. The length of a report will depend on the complexity of the harvesting area and the purpose of the document but those currently in the public domain commonly cover about 50 pages or 10 000 words. A further growing source of documentation on sanitary profile

studies derives from the US Clean Water Act TMDL investigations discussed in chapter 15 of this volume (Anon 2003; 2004; 2005; 2006).

The suggested contents list below is not prescriptive, rather it provides a template for adaption to the priorities for any specific profiling exercise.

1 **Executive summary**
   How was the work designed and executed?
   What were the principal findings?
   What are the implications for:
        shellfish growers;
        regulators and legislators;
        members of the public?

2 **Introduction**
   Purpose of the specific sanitary profile
        Why is it needed?
        What is the principal intended outcome?
        Who are the principal stakeholders?
   The legislative and policy context
   Overview of the growing area and its catchment context
        Legal extent of the harvesting area

3 **Pollution inventory (split by flow condition and season)**
   Point sources of:
        Treated sewage effluent
        CSOs and STOs
        Industrial effluents (e.g. pharmaceuticals and food processing)
        Quantification of point source flux
   Diffuse sources of catchment derived pollutants
        Land use survey
        Flux quantification from agricultural sources
   Direct inputs to the nearshore zone
        Boats
        Avian
        Aquatic mammals
        Animals grazing below Mean High Water

4 **Hydrographic and hydrological conditions**
        Nearshore hydrodynamics/tidal influences
        Patterns of point source inputs
        Diffuse episodic input locations/characteristics

Far-field effects from distant sources
Principal effects on fluxes quantified in 3

5 **Episodic effects/seasonal patterns impacts on**:
Compliance history
Input fluxes

6 **Management implications of 3–5**
In explaining compliance history
In designing remediation strategies
In defining feasible improvement potential

7 **Definition of empirical data gaps/requirements**
Further data acquisition
Reporting timescales
Resource implications
Implementation strategy

8 **Management structures**
Stakeholder engagement
Regulatory roles and responsibilities
Resource allocation and deployment

9 **Delivery of final management/action plan**
What is the intended outcome/aim?
When will new information be available?
What options will it facilitate?
Who will manage and take the key decisions?
When will the final plan be delivered?
When will the aims be delivered?

For reports intended to meet the aims of a statutory system, the final section will need to address the specific requirements of that system, e.g. recommended classification status, delineation of classified area or sampling plan.

8.5 SANITARY SURVEYS AND PATHOGENS

Sanitary surveys are currently primarily undertaken to identify sources of faecal indicator bacteria as the associated classification procedures are based on such organisms (see chapter 6). However, the intent of a shellfish hygiene programme is to reduce or, preferably, to remove the risk of human illness arising from consumption of the product. With respect to microbial contamination, it is

therefore necessary to consider how the likely pathogen content of potential sources of pollution and the subsequent fate in the environment with respect to the contamination of shellfisheries. In this regard, it is necessary to consider all microbial risks: bacteria, viruses and parasites (see chapter 3).

The limit of detection for some pathogens in seawater may be too low to detect levels that constitute a risk of infection. This problem is generally overcome if shellfish are used as the matrix for analysis due to the degree of concentration of such contaminants within the shellfish digestive tract. However, routine monitoring is not presently undertaken for most shellfish-associated pathogens. There is also the difficulty with some pathogens, such as noroviruses, in determining the significance of low levels in shellfish detected by real-time polymerase chain reaction (PCR). Risk assessment provides a means to supplement the information obtained from both faecal indicator and pathogen monitoring. However, the data necessary for full quantitative microbial risk assessment is generally not available.

Simple assessment procedures based on the principles of sanitary surveys have been undertaken to predict the risk of norovirus contamination in an area (Guilfoyle *et al*. 2007). Assigned risk was shown to correlate with the incidence of noroviruses in the areas as determined by real-time reverse transcriptase PCR. Similar approaches using different risk criteria, reflecting the potentially different sources, could be used for bacterial and protozoan pathogens.

8.6 SANITARY SURVEYS AS A PRECURSOR TO ESTABLISHING A SHELLFISH AQUACULTURE OPERATION

It is not possible to plan the location of wild harvest areas. However, this is possible with aquaculture operations. Location of such operations in areas impacted by faecal contamination, whether point source or diffuse, continuous or intermittent, will have potential negative impacts potentially including additional processing costs (such as for depuration) or, in the extreme, inability to harvest together with the possibility of outbreaks of illness affecting sales and, in the worst cases, additional controls or closure of the area. In addition, the classification of an aquaculture operation at the level that reflects such contamination will often cause friction between the operator and the authorities due to the effect on the business.

Lee *et al*. (2000) considered a simple approach to the siting of new shellfish aquaculture operations in England and Wales from a shellfish hygiene perspective. Essentially, they proposed undertaking a simple sanitary survey approach,

taking into account information from the relevant authorities on the location and nature of consented sewage outfalls and the results of any bacteriological monitoring of seawater or shellfish previously undertaken in the area, visually inspecting the site for other potential sources of contamination, and, if these aspects seem favourable, undertaking some preliminary bacteriological monitoring of the species intended to be grown. For the latter, it may be necessary to place shellfish in bags at a number of points in the vicinity of the intended aquaculture site. The operator may need to obtain advice from local or central authorities in the interpretation of this information. It must be stressed that the longer term monitoring undertaken by the authorities if aquaculture is progressed may still show the effects of sewage contamination (elevated concentrations of faecal indicator organisms) that had not been predicted by this simple assessment. However, the procedure should reduce the risks of investment from the shellfish hygiene perspective.

There is also the possibility that choices for the shellfish industry may arise from the conduct of sanitary surveys under official programmes. These surveys are usually intended to identify areas of potentially high faecal contamination within a harvesting area. However, the information contained in a report may be useful for the industry in identifying parts of an area that may be subject to the lowest levels of contamination. Where this is possible, this gives the industry the option of siting new operations or resiting existing ones in those locations. This has the potential to lead to better classification status if appropriately targeted monitoring is undertaken and/or a reduction in associated health risk. Care needs to be taken if different species are intended for new operations than those for which the sanitary survey was originally undertaken due to differences in uptake, retention and excretion of faecal indicator bacteria and the potential effects of differences in location, such as oyster trestles versus mussel lines.

8.7 CONCLUSIONS

Sanitary profiling is an emerging tool with both public health and regulatory management benefits. At one level, such reports provide a qualitative description of a harvesting area which is a useful baseline document defining characteristics, problems and potential solutions. However, there are approaches available, outlined in this chapter, to underpin semi-quantitative flux assessment for the faecal indicator bacteria. As developing regulatory agendas (driven in the EU by the Water Framework Directive and in the United States by the Clean Water Act) result in more abundant empirical data on faecal indicator flux and associated modelling tools, the quantitative component of such assessments

will become much stronger. In this mode, the sanitary profile will approach the information provision afforded by the Quantitative Microbial Source Apportionment studies reported in chapter 15. This is needed to underpin appropriate resource allocations to balance the diverse remediation options for sewage point source discharges and/or farming management practices to attenuate catchment-derived faecal indicator fluxes. Simple risk matrices using inputs from sanitary surveys, perhaps combined with some pathogen monitoring, may yield a better estimate of health risk than current classification schemes. Sanitary survey principles may also provide the shellfish industry with a tool for use in selecting locations for siting new aquaculture operations.

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9

Depuration and relaying

D. Lees, A. Younger and B. Doré

Bivalve molluscan shellfish feed by filtering large volumes of seawater and accumulating food particles from their surrounding environment. When that environment is contaminated by sewage, shellfish will also accumulate human pathogenic bacteria and viruses during filter-feeding and present a health risk when consumed raw or only lightly cooked. In order to render such shellfish fit for consumption three principal commercial treatment processes have been traditionally used. Firstly, heat treatment (cooking) can be used to destroy pathogens before consumption. Secondly, shellfish harvested from polluted areas can be replaced in clean areas (areas free of microbiological contamination) to allow shellfish to cleanse or purge themselves by continuation of their normal filter-feeding and digestive processes. This process is called 'relaying' or 'container relaying'. Thirdly, the 'natural cleansing' process can be performed in a controlled environment by immersion in tanks of clean seawater to allow sewage contaminants to be purged. This process is called 'depuration' or 'controlled purification'. The relaying and depuration processes, unlike cooking,

allow bivalve shellfish to be marketed as a live or fresh shucked product. This is commercially important for species such as oysters, clams and mussels which are traditionally eaten live or are lightly cooked prior to consumption. The terms depuration and relaying in the context of this chapter are considered to refer to removal of microbiological contaminants (bacteria and viruses) originating from sewage. The removal of heavy metals, pesticides, marine biotoxins and natural flora of shellfish, such as *Vibrio* spp., is not considered here. The term 'shellfish' used in this chapter refers exclusively to bivalve molluscan shellfish.

It is important to recognize that the most effective and reliable approach to controlling the microbiological contamination of shellfish is to harvest from areas with good water quality. The best practice approach is therefore for shellfish sanitation authorities to encourage, promote and strive to maintain an excellence of water quality in shellfish production areas. Encouraging commercial developments in such areas, rather than poorer quality areas, is also obviously an effective public health strategy. Unfortunately, however, the worldwide degradation of marine environments through discharges from human settlements and agricultural activities has lead to a shortage of pristine environments suitable for shellfish cultivation. Reduction of contamination through mollusc processing procedures is well known to be less effective than prevention of contamination by harvesting in high quality areas. However, processing provides a practical option for the many countries where waters are subject to sewage contamination. For bivalve molluscs sold live, depuration is often the preferred option and is practised extensively throughout the world including in Europe, North America, Asia and Australia. Relaying is also practised in some countries.

It is important that authorities and the shellfish industry understand the limitations of commercial decontamination procedures, basically that they have significant limitations for pathogen removal. Depuration and relaying should only ever be used in conjunction with harvesting area faecal pollution monitoring and grading programmes and should never be relied upon as the sole sanitation measure.

9.1 HISTORICAL PERSPECTIVE

The beginning of depuration as it is known today can be traced to the early part of the last century and was developed in direct response to a number of well publicised outbreaks of typhoid fever associated with shellfish consumption on both sides of the Atlantic. These outbreaks prompted investigations into methods of purifying contaminated oysters to render them fit for consumption. As early as 1911, studies in the United States demonstrated that it was possible to completely

eliminate coliforms from sewage contaminated oysters by relaying them in clean seawater (Phelps 1911). Similar work was carried out at the same time in the United Kingdom using mussels (Johnstone 1914). This work demonstrated a 93% reduction in levels of intestinal bacteria by taking mussels from a polluted harvesting area and relaying in cleaner (although not pristine) seawater. The potential of this work was seized upon by Dodgson in the United Kingdom who developed a depuration system for purifying mussels in Conwy, North Wales as early as 1915 (Dodgson 1928). This system made use of static seawater, which had been sterilised by sodium hypochlorite and subsequently neutralised by sodium thiosulphate, to purify mussels in large outdoor tanks. This system was still used to produce bacteriologically clean shellfish up until 1994. Similar systems were successfully set up in the 1920s by Wells in the United States to purify both oysters and clams (Wells 1926). Later in that decade ozone was introduced as a form of disinfection (Voille 1929) and this remains an important method for treating seawater in French depuration systems.

From the 1920s until the 1950s little progress was made in the development of depuration systems as the process fell out of favour with decreasing incidences of typhoid fever in the community and because of fears concerning the effect of chlorine on shellfish quality. The use of ultraviolet (UV) irradiation to sterilise seawater in depuration systems was introduced first in the United States (Arscisz and Kelly 1955) and then in the United Kingdom (Wood 1961). This novel treatment sparked renewed interest into improved designs of depuration systems and meant that it was now possible to use tanks of recirculating seawater with the water being sterilised on each recirculation by passing through the UV lamp. These systems had the advantage of being able to provide a flow of water through the shellfish to enhance filter-feeding whilst maintaining the microbiological quality of that water without the addition of by-products. This, allied to their versatility and simplicity, has meant that these systems have become the preferred option in several countries (Ayres 1978). More extensive accounts of the development of depuration can be found elsewhere (Canzonier 1991).

Depuration systems now in use worldwide include processes where water is static or changed in batches through to systems where seawater is flushed through continually or recycled through a steriliser. Water sterilisation processes include ozone, chlorination, UV irradiation and iodophores (Otwell *et al*. 1991; Roderick and Schneider 1994). Depuration has been applied to most bivalve shellfish species sold live including oysters, clams, mussels, cockles and scallops. Tank based depuration is now widely practised in many countries including Australia, France, Italy, Spain, the United Kingdom, and elsewhere. It is, however, now less widely used in the United States (Otwell *et al*. 1991).

Modern depuration systems have been shown on numerous occasions to reduce the levels of bacteria in sewage contaminated shellfish rapidly and efficiently. Therefore, shellfish treated in this manner normally easily comply with bacterial end-product standards (see below). Depuration, has thus, probably been instrumental in virtually eliminating sewage derived bacterial illness associated with shellfish consumption in the United Kingdom and other countries where it is extensively used (West and Wood 1985; Johnson *et al.* 1990). However, there remains a risk of viral illness following the consumption of depurated shellfish. Cases of viral illness have been recorded following the consumption of depurated shellfish which comply with legislative bacterial end-product standards (Gill *et al.* 1983; Johnson *et al.* 1990; Pontefract *et al.* 1993). This is discussed in more detail in section 9.4.2.

9.2 THE REGULATORY FRAMEWORK

The infectious disease hazards associated with consumption of bivalve shellfish have been recognized for many years. Consequentially most countries have enacted sanitary controls on the production and processing of bivalve shellfish. In the European Union (EU) these were drawn together into a European Directive 91/492/EEC (Anon 1991) to enable operation of the single European market in 1993. In 2006 European food safety legislation was renewed and the requirements contained in Directive 91/492/EEC were replaced by a suite of horizontal regulations. Those relevant to depuration are Regulation 853/2004 (Anon 2004b) covering requirements for food business operators including those operating depuration centres and undertaking relaying operations; Regulation 852/2004 (Anon 2004a) concerning general rules for the hygiene of foodstuffs including hazard analysis and critical control points (HACCP) requirements; and Regulation 2073/2005 (Anon 2005a) which sets out the microbiological criteria for foodstuffs. Also relevant for bivalve molluscs is Regulation 854/2004 (Anon 2004c) which sets out requirements for official control monitoring programmes which are the responsibility of the competent authority. In the United States, requirements are governed by interstate trading agreements set out in the National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish (Anon 2003). These regulations cover similar ground to EU requirements for clean growing areas, the controls and processing requirements for more contaminated areas, the hygiene conditions for processing and dispatch establishments, requirements for marketing documentation and other relevant issues. Third country imports into the EU and United States have to be produced to the same standard as domestic products.

9.2.1 Ascribing a pollution limit for shellfish to be depurated or relayed

It has been accepted for many years that the depuration and relaying processes are only partially effective control measures. In particular, it is critical that they are not used in attempts to rectify the effects of excessive pollution. For this reason a key feature of both EU and United States legislation is the setting of a pollution cap or limit above which shellfish are not permitted to be depurated or relayed. Both EU and United States sanitary legislation requires compliance with this pollution limit to be determined for each harvesting area through the use of faecal indicator (*E. coli* or faecal or total coliforms) monitoring. Faecal indicator monitoring determines the appropriate treatment in accordance with the level of contamination and the prescribed statutory standards.

In the EU, faecal indicators are measured in shellfish flesh and intravalvular liquid whereas in the United States indicators are measured in the shellfish growing waters. Both the EU and the United States systems base standards on a 5-tube 3-dilution most probable number (MPN) test. In the EU, *E. coli* was adopted as the sole faecal indicator in the revised regulations introduced in January 2006. These regulations also introduced a required standard reference method for *E. coli* analysis for bivalve shellfish measured either in the final product (Anon 2005a) or in harvesting areas during official control monitoring programmes (Anon 2005b). In the United States either faecal coliforms or total coliforms may be used and the required methods are those set out in the NSSP Guide for the Control of Molluscan Shellfish (Anon 2003). US Food and Drug Administration (US FDA) 'approved' and EU 'category A' standards describe the cleanest growing areas from which shellfish can be taken for direct human consumption without further processing. All shellfish from EU category A areas must contain less than 230 *E. coli* in 100 g of shellfish flesh. The US FDA programme gives a choice of using either total or faecal coliforms to establish a classification. It further expresses standards in two components, a geometric mean (GM) count of results and an upper standard which not more than 10% of results can exceed. Approved areas must comply with a total coliform GM of 70 per 100 ml water with not more than 10% of samples exceeding 230 per 100 ml. Alternatively they can comply with a faecal coliform GM of 14 per 100 ml water with not more than 10% of samples exceeding 43 per 100 ml.

In both EU and United States legislation shellfish cannot be harvested for direct human consumption from growing areas exceeding the above levels of contamination. They may however be placed on the market following commercial depuration, relaying or heat processing. However, because these processes are known not to be effective if shellfish are excessively polluted an

upper threshold is placed on the degree of contamination beyond which such procedures may not be used. Shellfish from EU 'category B' and US FDA 'restricted' classifications may be placed on the market following depuration or relaying. Shellfish from EU category B may also be heat treated by a permitted method (Anon 2004b). EU category B areas must contain less than 4600 *E. coli* per 100 g of shellfish flesh and intra-valvular liquid in 90% of samples with an upper limit of 46,000 *E. coli*/100 g for all samples. US FDA restricted areas must comply with a total coliform GM of 700 per 100 ml water with not more than 10% of samples exceeding 2300 per 100 ml. Alternatively they can comply with a faecal coliform GM of 88 per 100 ml water with not more than 10% of samples exceeding 260 per 100 ml.

In EU legislation shellfish from harvesting areas exceeding the limits of category B may only be placed on the market following protracted relaying (a minimum period of two months is specified unless a risk assessment can justify a shorter period) (Anon 2004b) or commercial heat treatment by an approved method. Shellfish from category C areas must contain less than 46 000 *E. coli* per 100 g of shellfish flesh. Relayed shellfish may, if necessary, be depurated before being placed on the market. US FDA controls do not incorporate an equivalent to EU category C. Bivalve shellfish growing areas exceeding these prescribed levels of contamination, or areas for which harvesting area survey and classification has not been conducted, cannot be harvested for human consumption in either United States or EU legislation.

If areas meet the above standards only for certain periods because of predictable pollution events authorities may classify them for a restricted period. In the US FDA programme such areas are defined as 'conditionally approved' or 'conditionally restricted' and may be harvested during periods when they meet the standards subject to a management plan. Such arrangements may also apply in the EU although they are not formally defined by the legislation.

A key consideration for ascribing the above pollution thresholds is that faecal indicator methods should be sensitive and suitable for the task of recovering faecal bacteria damaged by exposure to seawater. It is well established that methods such as direct plating onto a hostile media (McConkey agar for example) result in low faecal indicator recovery and thus undermine the public health protection afforded by this requirement. For this reason both EU and United States regulations stipulate the faecal indicator methodologies that should be used. In both cases broth cultures in a MPN format are specified. In the United States approved American Public Health Association (APHA) or other methods are stipulated in the NSSP Guide for the Control of Molluscan Shellfish

(Anon 2003). In the EU, a reference method (ISO 16649-3) for *E. coli* analysis in shellfish is now specified in the legislative requirements (Anon 2005a and Anon 2005b). Unfortunately, commercial pressures, the ease of direct plating methods in the laboratory, and a failure to appreciate the significance of the methodology stipulations, has meant that these safeguards are not always complied with in practice. A priority for enforcement agencies should be to ensure laboratory compliance with the stipulated methods to ensure achievement of this basic public health safeguard.

9.2.2 Regulation of the depuration and relaying processes

Commercial depuration and relaying, when used as treatment processes to reduce microbial contaminants, are subject to legal control in several countries including those in the EU and the United States. The NSSP Guide for the Control of Molluscan Shellfish (Anon 2003) sets out requirements in the United States Regulation and 853/2004 (Anon 2004b) sets out requirements in the EU. The EU Regulations detail a number of requirements for the construction and general running of purification centres. These cover elements such as tank construction and operation, the operation of batch systems and non-mixing of species during purification. As far as the purification process itself is concerned, three essential features are identified and these are that shellfish should rapidly resume filter-feeding activity, remove sewage contamination and not become recontaminated. Also covered are requirements for hygiene of premises, the approval of shellfish purification and dispatch centres, laboratory testing, packaging, labelling, transportation, wet storage, and movement documentation/ traceability requirements. Requirements that apply to relaying operations are also covered in detail.

In the EU, in addition to the above physical and environmental requirements, purified shellfish are required to comply with an end-product standard for shellfish sold live which includes the faecal coliform parameter of less than 230 *E. coli* in 100 g of shellfish flesh (Regulation 2073/2005). The regulatory principles relating to purification plant construction and operation set out in the EU Directive are implemented by the 'competent authority' in each member state. In practice, compliance with the end-product *E. coli* standard is frequently seen as evidence of satisfactory design and operation of depuration plants. However, evidence from numerous sources suggests that depuration plants functioning satisfactorily and achieving faecal indicator criteria may still fail to remove human enteric viruses. Perhaps most significant is the epidemiological evidence demonstrating that infection can occur following consumption of

depurated shellfish that comply with the faecal indicator standards. Removal of pathogens, including viruses, during the depuration and relaying processes is more fully considered in section 9.4.

A key consideration for maximizing the health protection that can be afforded by the depuration and relaying processes is that they are operated according to principles of best practice and not according to simple compliance with faecal indicator standards. Indeed, a formal advisory report from the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) in 1999 advised the European Commission that the conventional faecal indicators are unreliable for demonstrating the presence or absence of human enteric viruses (norovirus) and that the reliance on faecal bacterial indicator removal for determining shellfish purification times was unsafe practice (Anon 2005a). Consequently it is strongly recommended that criteria for the operation of shellfish depuration and relaying are based on optimisation of the process for most effective pathogen removal. This necessarily considers aspects such as the quality of the animals prior to initiation of the process, how to maintain optimised physiological conditions for the animals, the quality of the seawater, the design of systems to separate and remove faecal material excreted by shellfish. These factors are further considered in section 9.3. The target of compliance with faecal indicator standards post depuration may seem an attractive, and simple, alternative to such detailed considerations. However this approach will not maximise the available public health protection from these processes. Indeed, taken to extreme, this approach can lead to dangerous practices such as extremely short depuration times (such as four hours). Consideration of the whole food process is of course the key principle of Hazard Analysis Critical Control Point (HACCP) analysis. The application of HACCP to shellfish depuration and relaying is considered in section 9.5.

9.3 PRINCIPLES AND PRACTICE OF THE DEPURATION PROCESS

9.3.1 Basic principles

The depuration process normally involves placing trays of shellfish into a purpose-made tank which is then filled with clean seawater or seawater treated to ensure cleanliness. The water is then recycled through the system, usually via sterilising equipment, and then returned via a cascade or spray bar to allow sufficient aeration of the water for the shellfish to function normally. Alternatively, systems are operated on a single pass flow-through basis. Given the correct physiological conditions, shellfish will resume normal filter-feeding

activity and excrete contaminants in their faeces. The faecal material so produced should be allowed to settle to the bottom of the tank and then be removed at the end of the cycle.

For depuration to be effective in removing microbiological contamination, the design of the system and the operation of the entire process must allow shellfish to:

- rapidly resume normal filter-feeding activity and to maintain this for the duration of the process. This requires optimization of physiological conditions;
- facilitate removal and separation of faecal contaminants excreted by shellfish. This requires appropriate design and operation of systems; and
- avoid any contamination or re-contamination of the shellfish during the process. This requires an appropriate quality of seawater used in the process and proper operation of the system.

To ensure these requirements are met, the design and operation of purification systems must be carefully documented and controlled. It is highly recommended that purification systems are subject to a formal approval process by the appropriate local regulatory authorities. The approval should be a detailed document describing the approved plant and setting out the various operating conditions and criteria for successful depuration, in effect a HACCP type approach. The criteria are discussed in more detail below.

9.3.2 Suitability of shellfish intended for depuration

Live bivalve molluscs that are to undergo depuration effectively must be in good condition. They are sensitive animals that are susceptible to temperature extremes and physical shock. It is therefore vital to ensure that good harvesting and general handling practices are followed so that the animals are not unduly stressed.

At all times post-harvesting, the re-immersion of live bivalve molluscs (other than during depuration or controlled immersed storage) should be avoided. In any event, immersion should never take place in water of inferior quality to that from which the shellfish originated. Shellfish, when immersed, will normally open and recommence filter-feeding and may accumulate any contaminants which may be present in the surrounding water.

Before they are loaded in the depuration tank, shellfish should be washed and culled (the process of separating dead or broken shellfish, and other species, from the live, intact shellfish).

Any batch of shellfish undergoing purification must be of the same species and from the same class of production area. Whilst most shellfish can be harvested by mechanical means, cockles (*Cerastoderma edule*) have been shown to exhibit high levels of mortality under depuration conditions due to the damage and general stress caused by such practices, in particular by suction dredges (Boulter *et al.* 1994).

9.3.3 Physiological parameters

In order that normal filter-feeding may take place and to avoid mortalities, it is essential to create the correct physiological conditions for the shellfish being depurated and these are outlined below.

9.3.3.1 Dissolved oxygen

To facilitate normal shellfish activity, sufficient oxygen must be available in the water. As a general guide, minimum dissolved oxygen levels of 50% saturation are recommended for purification systems. In re-circulating systems the dissolved oxygen content of the water can be affected by a number of factors such as water surface area to volume ratios; flow rates; shellfish to water ratios; seawater temperature; the metabolic rate of shellfish under purification (which may be environmentally and/or genetically determined); seawater salinity and the method of aeration used in the system. All these factors must therefore be carefully controlled during the purification process. The method of aeration must not disturb the normal activity of the shellfish or the settlement of shellfish faecal material. In addition, the presence of small gas bubbles in the water may inhibit respiration of the shellfish by blocking gas exchange in the gill tissue. Primary aeration is normally by means of a cascade but supplementary aeration may be added by using air diffusers placed in the bottom of the tank or sump provided such aeration does not disturb the molluscs or the settling of faecal material. The air supplied must be clean and free from oil. Centrifugal pumps are therefore recommended.

9.3.3.2 Loading

Shellfish must be loaded in the trays at a density that allows them the room to be able to function normally. They should be able to open as they would in the natural marine environment and carry out their normal filter-feeding activity. This loading arrangement will vary according to the species of shellfish being depurated. Mussels (*Mytilus edulis*) for example are able to function in deeper layers than are native oysters (*Ostrea edulis*) which are depurated in trays as

a single layer. The acceptable tray loading densities for each species should be defined for each system as part of the approval or HACCP process.

The level of water above the shellfish should also be sufficient to ensure that the shellfish remain immersed throughout the entire period of depuration. Mussels often move upwards in the trays during the process by attachment of their byssal threads to the side of the trays. A greater depth of water above the uppermost tray of mussels is therefore required (8 cm is considered sufficient in the United Kingdom). Other species are more sessile and consequently do not need to be immersed to such a depth (3 cm is specified in the United Kingdom). The trays of shellfish within a system need to be arranged in such a way as to ensure that water cannot "short circuit" around them. Therefore trays are normally orientated so as to provide a complete barrier to flow. In this way, water must pass through the trays of shellfish (providing oxygen and dispersing metabolic by-products as it does so) before it can be re-circulated back through the system.

9.3.3.3 Shellfish to water ratio

The loading of shellfish for a given volume of water needs to be controlled, both to maintain dissolved oxygen levels to ensure optimum shellfish activity and also to ensure that the build-up of metabolic by-products does not reach inhibitory levels. The maximum shellfish capacity should therefore be specified in the approval conditions of each type of system. This will be dependent upon the type of system and the individual species concerned.

9.3.3.4 Water flow

It is essential to provide a sufficient and even flow of water throughout the system to maintain adequate levels of oxygen in the water and prevent the build-up of metabolic by-products which may inhibit normal shellfish activity. The flow of water must not, however, be so great as to prevent the settlement of faecal material or cause the disturbance of such material that has already reached the bottom of the tank.

9.3.3.5 Salinity

It is also necessary to provide seawater of the correct salinity range for the shellfish being depurated, and to take account of the salinity of the harvesting areas, as requirements vary according to species. As an example the species commonly depurated in England and Wales generally occur in shallow coastal waters which are subject to freshwater influences from rivers, streams and general land-run-off. Consequently the salinity of water experienced by the majority of

shellfish is usually less than the normal seawater maximum for the United Kingdom of 35‰. The minimum allowable salinities for each species should be specified as part of the approval conditions. In the United Kingdom best practice guidance recommends that the salinity of seawater used during depuration should be maintained within ±20% of that found in the harvesting area. It should be noted that the solubility of oxygen in seawater decreases with increasing salinity. Artificial seawater may be used where access to a ready supply of suitable natural seawater is not available. This is made up from a standard mix of five basic salts to the required salinity using fresh water of potable water quality.

9.3.3.6 Temperature

The solubility of oxygen in seawater decreases with increasing temperature. The metabolism of shellfish is directly affected by the temperature of their environment. With decreasing temperature, shellfish become less active and contaminant removal, as a result, is decreased. Temperature is particularly important for effective removal of viral pathogens and is considered in more detail in section 9.4.2. Therefore water temperatures are required to be kept above a minimum level during depuration and these should be specified as part of the approval conditions. However, if the temperature becomes too high, then the dissolved oxygen level in the system may fall (if the flow rate and method of oxygenation are not sufficient to maintain it) again leading to a cessation of activity and potentially also shellfish mortalities. In addition, high temperatures during the correct season (summer) may cause condition-ready shellfish to spawn causing the release of gametes into the water column. This is likely to cause a significant increase in the turbidity of the water which in turn will reduce the efficiency of the UV disinfection system. The shellfish themselves are also likely to be weakened by the process of spawning and consequently their depuration efficiency may be affected.

When a depuration system is first filled with seawater, care should be taken to ensure that the water is not significantly warmer or colder than the temperature to which the shellfish have become accustomed. Failure to take this into account could lead to temperature shock inducing either spawning or undue stress, thus reducing shellfish activity.

9.3.3.7 Turbidity

Control of turbidity is important for two reasons. Firstly, for UV systems, disinfection effectiveness is considerably reduced by turbidity (Qualls *et al.* 1983). Thus, shellfish contamination may occur from insufficiently disinfected seawater. Other seawater disinfection systems may also be adversely affected by turbidity.

Secondly, if the turbidity is excessive then the gills of the shellfish may become clogged, again preventing effective depuration. Thus requirements for seawater turbidity limits should be specified as part of the process approval conditions.

9.3.3.8 No disturbance

In addition to the above, it should be noted that shellfish are sensitive creatures and if disturbed directly by the effects of cascades, aeration or operator handling during the purification cycle, will cease to function effectively. Aeration cascades should not therefore fall directly onto shellfish and shellfish should not be disturbed during the process cycle.

9.3.4 Parameters to ensure effective decontamination and avoid re-contamination

9.3.4.1 Physical environment

Basic hygiene of the premises must be observed to ensure that contamination does not take place. Tanks should be housed in a building with a roof to prevent aerial contamination from birds. Vermin such as rodents should also be excluded from the area.

9.3.4.2 Drain down

During depuration, contaminants are excreted as part of the digestive process, predominantly in the form of mucoid faecal strands, which must be allowed to settle to the bottom of the depuration tank. Once settled, re-suspension of this faecal matter must be avoided as this may lead to its re-ingestion by the filter-feeding shellfish.

   At the end of the cycle seawater in the system should be drained down below the level of the shellfish before they are removed. This prevents turbulence caused by removal of trays of shellfish immersed in water leading to the possible re-suspension and re-ingestion of faecal material in neighbouring shellfish. At the end of each cycle, the remaining water must be discarded and the bottom of the tank thoroughly cleaned, as this is where the shellfish faecal material containing the contaminants will be concentrated.

9.3.4.3 Use of a batch system

In order to avoid re-contaminating the shellfish during the process, it is vital that all steps should be taken to avoid the possibility of re-suspension and therefore

re-ingestion of shellfish faecal material. One of the most important practices in this regard is the operation of a batch system, that is once the tank has been appropriately loaded and the cycle has commenced, no additional shellfish should be added or removed until the full cycle has been completed and the tank drained down. If this practice is not followed then re-contamination, either from added shellfish or by re-ingestion of re-suspended shellfish faecal material caused by trays being removed whilst still immersed, may occur.

9.3.4.4 Seawater quality and recycling

Good quality intake water is vital to avoid the possibility of contamination, or re-contamination, during the process. EU Regulation 852/2004 defines clean seawater as being: 'natural, artificial or purified seawater or brackish water that does not contain microorganisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food' (Anon 2004a). The lack of any defined values has caused some practical problems with the interpretation and implementation of the requirement for clean seawater to be used.

   If treatment of the seawater is necessary, then the authorities should verify the treatment method and authorize its use as part of the approval process for the system. If the purification system is recycling water then steps must be taken to ensure that the recycled water is of adequate quality. Features vital in this respect are some form of in-line disinfection system (UV is often used) and adequate provision for the settlement of shellfish faecal material. Disinfection systems used for seawater are further discussed in section 9.3.7.

9.3.5 Design of depuration systems – typical system operation

Figures 9.1 and 9.2 illustrate the basic layout for a purification system. Water is pumped from a storage tank via a UV unit (or other form of water disinfection process) and then aerated by way of a cascade or spray bar. This normally comprises a length of pipework drilled with holes to produce a number of even jets of water. The impact of these jets on the surface of the water already in the tank provides the level of oxygenation required for normal shellfish activity, providing a sufficient flow rate of water through the system is maintained. Most systems are designed to operate at one flow rate which is calculated to allow a sufficient safety margin to account for any decrease in the oxygen content of the water due to temperature increases, provided that they are within reasonable limits. Shellfish may be able to depurate effectively at higher temperatures if adequate oxygen levels can be maintained and the animals are not stressed.

Higher temperatures are required for effective removal of viral pathogens – see section 9.4.2.
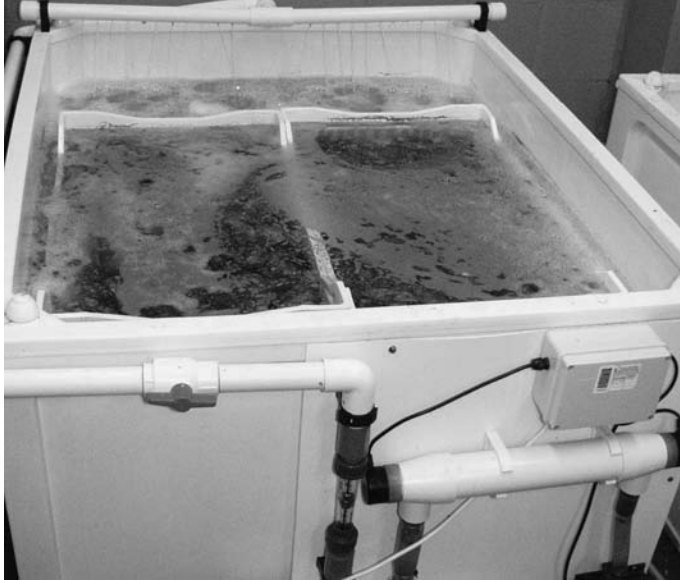
Figure 9.1 Small scale shallow tank depuration system ©Cefas.
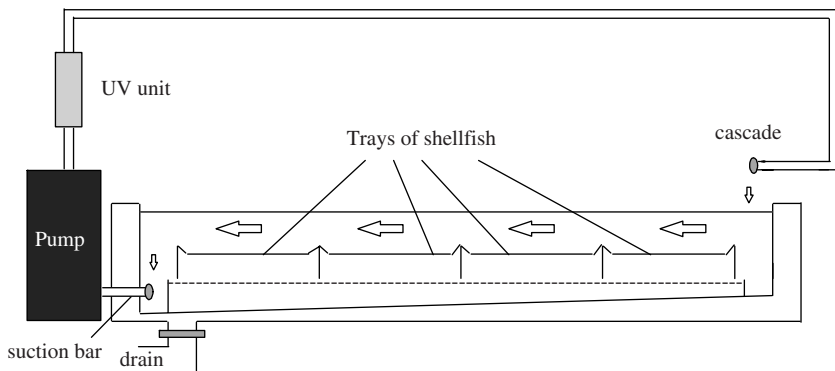
Figure 9.2 Shallow tank system ©Cefas.

An adequate and even flow of water through the tank is essential to ensure that all the shellfish in the system receive well oxygenated water and that there is not a localized build-up of shellfish metabolic by-products that may inhibit shellfish activity. Water passes through the trays of shellfish (the trays are perforated to allow through-flow of water) and is then removed from the tank via the suction bar for recirculation through the UV disinfection system and back again into the tank via the cascade.

At the end of the purification cycle, water is drained from the system in a controlled manner, thus avoiding the re-suspension of sedimented shellfish faecal material. This is normally achieved by using the recirculation route which maintains the same flow rate and direction. Once the level of the water has fallen below the lowermost shellfish, they can be removed and the remaining water in the tank, containing the sedimented shellfish faecal material, discarded. In the United Kingdom tanks are designed so that this residual water constitutes 10% of the total water held in the system. The base of the tank is normally designed to include a 1:100 slope towards the drain to facilitate cleaning.

9.3.6 Design of depuration systems – some example systems in use in the United Kingdom

9.3.6.1 Shallow tank

The first shellfish purification tanks to be used in the United Kingdom were of the shallow tank design in which trays of oysters and clams were stacked up to three layers high (Figure 9.2). The stacking of mussels in this type of system was not permitted due to their higher level of metabolic activity and the limited degree of oxygenation achievable in this type of system due to the relatively low flow rates involved (normally one exchange of all the seawater in the system per hour).

The need to purify large quantities of mussels therefore resulted in large shallow tanks that, due to their size, were often sited outdoors. This meant that they were exposed to the elements making temperature control of the process difficult and making them vulnerable to aerial contamination. A more recent development has been the small scale shallow tank (Anon 1995a) which has been shown to be able to successfully depurate shellfish, including mussels stacked up to three layers high with a flow rate of 20 litres/min or two complete changes per hour (Boulter 1992). This system has a nominal capacity of 90 kg mussels or 990 oysters and approximately 600 litres of seawater. Its relatively compact size means that it can be comfortably housed within premises as small as a garage and it is relatively cheap, making it popular with the small-scale operator.

9.3.6.2 Multi-layer system

The use of the multi-layer system enables trays of shellfish to be stacked up to six layers high (Anon 1995b; Anon 1995c) (Figure 9.3). This is an advantage with the lower value shellfish such as mussels where a high density load is more economical. It has the additional advantage of saving a significant amount of floor space compared to the shallow tank system. The multi-layer system has a relatively high shellfish to water ratio and consequently the flow rate needed to maintain an adequate level of dissolved oxygen in the circulated water is relatively high. The required flow rates for the medium and large scale multi layer systems are 12.5 m^3 (208 litres/min) and 9.5 m^3 (158 litres/min) per hour respectively which is equivalent to five complete exchanges of seawater per hour.
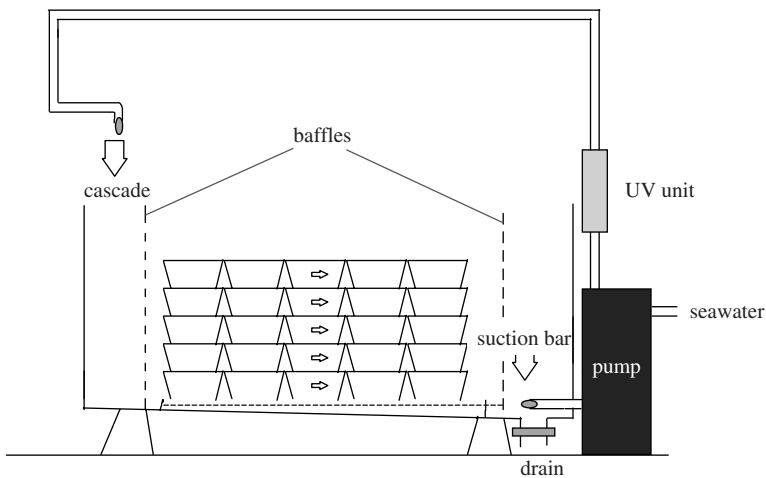
Figure 9.3 Multi layer system ©Cefas.

In these systems baffles are necessary, due to the high flow rate involved, to maintain an even flow of water through the system. There are normally two baffles per tank, one immediately after the cascade and the other close to the suction bar. A typical baffle consists of a sheet of plastic evenly drilled with holes through which water is allowed to pass. In the United Kingdom there are 750 (medium scale) and 1500 kg (large scale) (nominal mussel loading capacity) versions of this type of system.

9.3.6.3 Stack system

The vertical stack systems (Figures 9.4 and 9.5) used today were developed in the 1960's. Space saving was again the advantage over the traditional shallow

tank system with the additional benefit of ready access to individual containers without the need to drain down the entire system as is the case with all other systems commonly in use (Anon 1995d). However, this type of system has the disadvantage of being relatively expensive and consequently its use has generally been limited to high value molluscs such as clams and oysters. Such systems generally have a nominal capacity of 2000 oysters using around 600 litres of seawater. The required flow rate for this type of system is 15 litres/min.

Figure 9.4 Small-scale vertical-stack depuration system ©Cefas.

9.3.6.4 Bulk bin

The bulk bin system (Figure 9.6) is designed specifically for the purification of mussels which, historically, were purified in trays in layers up to 8 cm deep in shallow tank systems. Large tanks up to 120 cm deep have been used for the degritting of mussels in the Netherlands for some time and the Sea Fish Industry Authority (Anon 1995e) undertook some development work to produce a system capable of purifying mussels, based on the Dutch conditioning systems. The main concern was to provide sufficient flow of water to maintain the required levels of dissolved oxygen and to ensure a sufficient down-welling of water to take the egested faecal material to the bottom of the tank and to avoid its re-ingestion by
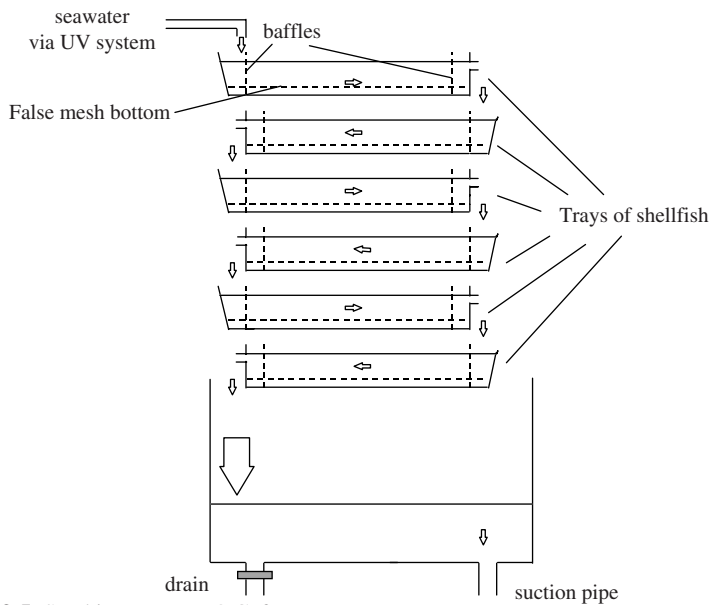
Figure 9.5 Stacking system ©Cefas.

the shellfish. The result was a system that can contain many individual units linked in series. The individual units are plastic pallet bins which can be easily handled. For large quantities of shellfish this type of system has the advantage of low capital and labour costs through mechanised handling. In addition, units can be added or removed, as appropriate, to cope with different sized batches.
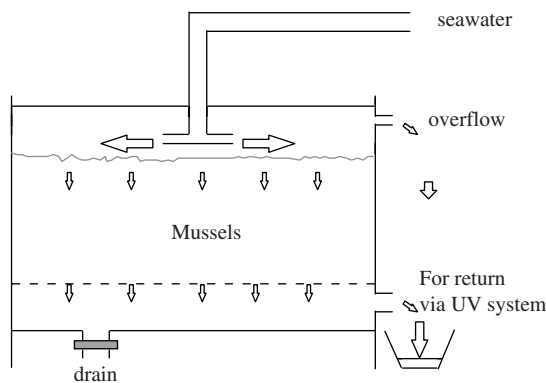
Figure 9.6 Bulk Bin System ©Cefas.

9.3.7 Seawater disinfection

There are presently four main options with regard to the disinfection of water entering and re-circulating within depuration systems. These are chlorination, UV light, ozonation and iodophors.

9.3.7.1 Chlorination

Chlorination was the first form of disinfection to be used in depuration systems in 1914 (Johnstone 1914). The disinfection capability of chlorine (a powerful oxidising agent) is well known, although bacteria are more susceptible than enteric viruses. The use of chlorine in shellfish purification can have a number of drawbacks if the disinfection process is not carefully monitored and controlled. Levels of free chlorine as low 0.2 ppm have been shown to have an inhibitory effect on the activity of oysters (Dodgson 1928; Hedstrom and Lycke 1964). The chlorinated water must therefore be dechlorinated using sodium thiosulphate, activated charcoal and/or vigorous aeration before it is allowed to come into contact with the shellfish. There is evidence that sodium thiosulphate may also have an adverse effect on the normal functioning of the shellfish (Kelly 1961) possibly due to the fact that the process of dechlorination depletes the oxygen content of the water. Furthermore, shellfish that have been depurated in systems using chlorine disinfection may exhibit chemical tastes and a chewy texture (Voille 1929). In addition, the use of chlorine can give rise to a number of chlorinated by-products of which the short- and long-term effects are not fully understood. Chlorine disinfection has given way in many countries to the use of UV irradiation or ozonation.

9.3.7.2 Ultraviolet irradiation

Ultraviolet irradiation is the preferred means of disinfection for purification systems in several countries. It has an advantage over other means of disinfection in that it does not alter the physical or chemical properties of seawater. The UV systems commonly used consist of a UV tube, filled with gas and mercury, housed inside a quartz sleeve. Water is passed parallel to the tube within a jacket of stainless steel or PVC. Power is supplied to each end of the lamp causing an arc which ignites the gas, producing a mercury vapour. Low pressure mercury vapour lamps produce 85% of their light in the UV C range at 253.7 nm (Herrington 1991) which is the wavelength at which peak germicidal activity of UV light occurs. The UV light causes disruption of the DNA or RNA of the microbial cell which usually leads to lethal changes in the biochemical processes. Clearly, only the water passing through the UV unit is subjected to the disinfective action of the UV light

and those organisms held within the shellfish will not be affected, as this form of disinfection, unlike chlorination, has no residual effect.

The unit of measurement (or dosage) for UV disinfection is the microwatt second per square centimetre (μws/cm^2 – equivalent to $1/1000$ mJ/cm^2). This unit of measurement is effectively the UV intensity multiplied by the contact time. The effect of UV varies according to the particular organism concerned. Inactivation of hepatitis A (HAV) may require a dosage in excess of 40 000 μws/cm^2 whereas coliforms require 6000 μws/cm^2. In England and Wales the minimum required dose rate is currently 10 000 μws/cm^2.

UV irradiation has the disadvantage of not being able to penetrate very far into water and is most effective at distances of less than 25 mm. Furthermore, its transmission and therefore its bactericidal action is greatly reduced by colour, dissolved iron salts and relatively low levels of turbidity, thus water with a turbidity as low as 5 NTU can reduce the UV disinfection efficiency by 90%. Particulate matter in the water effectively acts as a shield behind which micro-organisms can escape the effects of the UV light. For UV disinfection systems it is therefore important to specify and control the maximum turbidity of seawater permitted in order for disinfection to be effective.

The UV lamp should be changed before the end of its rated life and the protective quartz sleeve regularly cleaned as this is prone to the build-up of slime which will block the passage of UV light. Work by Souness and Fleet (1979) has shown that the build-up of UV resistant species of bacteria can occur in treated water. This could constitute a health risk should significant numbers of such organisms proliferate.

9.3.7.3 Ozonation

Ozone is a strong oxidizing agent and was first used in depuration systems in France in 1963. A number of depuration systems in France use ozone for seawater sterilization, it is also used for some plants in Australia and elsewhere. Unlike chlorine, ozone has the advantage of not imparting taste or odours to the shellfish and neither is the appearance affected. Effectiveness of ozone can be influenced by changes in temperature and pH. Experiments have shown that there is an increased biocidal effect at pH 7.2 as opposed to 5.9 and 4.3 respectively (Englebrecht and Chain 1985). However, the initial cost for ozonation equipment is generally high compared with chlorination and UV disinfection, but this may be offset by lower running costs (Blogoslowski *et al.* 1976). As is the case for chlorination, ozonation takes place in a contact chamber outside of the main depuration tank. Residual ozone is removed by degassing using compressed air which gives ozonation the additional benefit of increasing

the oxygen content of the water. The dosage of ozone should not exceed 0.5 ppm as by-products such as hypobromous acid may inhibit shellfish activity (Blogoslawski *et al.* 1976).

9.3.7.4 Iodophors

Iodophor disinfection in depuration systems has been carried out in Italy. Re-circulating systems using 0.1 to 0.4 mg iodophor/litre of tank water produced rapid reductions in the bacterial content of the shellfish without unduly affecting the activity of the shellfish or their organoleptic quality (Fleet 1978). However iodophors are not in common use in shellfish purification systems.

9.3.8 Commissioning and testing new systems

As part of the approval process for a new system it is recommended that microbiological commissioning tests be undertaken. Generally this is performed by fully loading a system with shellfish to its maximum capacity and undertaking a full depuration cycle. Pre- and post-depuration samples of shellfish are taken from a variety of locations within the system and tested for *E. coli*. Reasonably high levels of contamination are required in shellfish prior to depuration (such as > 2000 *E. coli* per 100 g) to ensure a representative test. All normal physiological parameters (including temperature, oxygen levels, shellfish mortality rates) are monitored to ensure compliance with the determined criteria. Reduction of *E. coli* to well below the end-product standard (230 *E. coli* per 100 g) should be demonstrated and ideally complete elimination of *E. coli* should be achieved. In the United Kingdom depuration plant commissioning tests are acceptable if they reduce *E. coli* levels of circa 4600 *E. coli* per 100 g to < 80 *E. coli* per 100 g (Anon 2007). If plants fail to achieve this criterion it may indicate an operational or design problem and a re-test is recommended. Water disinfection systems should routinely produce seawater showing absence of faecal indicators in 100 ml.

9.3.9 Microbiological criteria for ongoing compliance testing

It is well recognized that the implementation of a HACCP procedure, as required by European and United States regulations (see chapter 10), is the best approach to control of the functioning of the whole depuration system. Microbiological checks should be incorporated as one element of a HACCP approach. It is however important to avoid over reliance on microbiological testing as an

indicator of satisfactory plant performance considering the poor effectiveness of *E. coli* testing in relation to viral pathogen removal (see section 9.4.2). Well run and optimized depuration systems should be routinely capable of achieving absence, or low numbers, of *E. coli*. Systems which routinely give counts close to, or above, the *E. coli* end-product standard (230 *E. coli* per 100 g shellfish flesh) are unlikely to be optimized for best performance. Alternatively shellfish may have been too highly polluted prior to depuration. Depuration plant operators should routinely monitor the effectiveness of their system through microbiological checks performed on shellfish both before and after depuration and through checks on the seawater. In EU legislation, all shellfish that are intended for direct human consumption must meet the end-product standard. This includes shellfish taken directly from a class A area and also shellfish that have been depurated in an approved shellfish purification system. The microbiological aspects of the end-product standard requires shellfish to comply with <230 *E. coli* per 100 g shellfish flesh and intra-valvular liquid and the absence of *Salmonella* in 25 g.

However, it is well recognized that the majority of shellfish-associated food-poisoning outbreaks are due to viral pathogens (see chapter 3) and that *E. coli* is a poor indicator of viral presence in shellfish particularly after depuration (Lees and Doré 1995). Unfortunately, there is at present, no validated test that can be routinely used for verification of end-product quality with regard to viral contamination. This makes it even more important that the approval of purification systems is based on a HACCP approach, which considers operation of the whole system, and not merely on meeting end-product microbial standards.

Recent research has highlighted the parameters particularly significant for virus removal during depuration (section 9.4.2). It is important to fully consider these issues when setting operational parameters for shellfish depuration systems.

9.3.10 International perspective

Up-to-date information on depuration practices in different countries is hard to obtain. This section provides an overview from available published information and personal communication to the authors. It is not intended to be a comprehensive review of depuration practices internationally but serves to highlight some of the differences or similarities between countries. If readers require definitive information they should contact the regulatory authorities in the country of concern.

In Australia the major species depurated is the Sydney rock oyster (*Saccostrea commercialis*), largely produced in New South Wales. Plants in use include the pool type, usually of masonry construction and similar to United

Kingdom shallow tank systems; high density tray types, several shallow trays stacked on top of each other (similar to United Kingdom stack systems); and stacked box, similar to low volume version of high density tray system. UV sterilizers with recycling process waters are widely used in these systems. Critical Hazard Analysis Rating (CHAR) based on the HACCP concept has been designed for the practical application of the HACCP type approach in monitoring oyster purification with a wide variety of factors (oyster quality, water quality, plant operations, plant design, hygiene, records and product identification and construction) contributing to the assessment of hazard. In the United States, the NSSP requires that a comprehensive study of the effectiveness of the depuration process at a particular plant is carried out before it can be used commercially. A HACCP study must be carried out to determine the Critical Control Points at a particular plant and its operations. The required operating parameters and criteria are set out in the NSSP Guide for the Control of Molluscan Shellfish (Anon 2003). Minimum depuration periods are set and the salinity of the process water is also controlled. Criteria for UV disinfection is established along with specified faecal indicator kill rates for approving systems.

In the United States, there are fewer depuration plants operational now than previously, with a greater reliance placed on harvesting shellfish from cleaner areas, thus avoiding the need for depuration. Those in operation process hard shell clams or quahogs (*Mercenaria mercenaria*), soft shell clams (*Mya arenaria*) and Eastern oysters (*Crassostrea virginica*) (Somerset 1991). Seawater is obtained either from appropriate production areas or from saltwater wells. Both re-circulating and flow-through systems are used depending on the quality of the water available.

France is a major user of depuration within Europe with many systems in use. Either ozonation, chlorination or UV light are (or have been) used for the disinfection of process water depending on the type and size of plant. The main species of commercial interest are mussels (*Mytilus edulis* and *M. gallopro-vincialis*), oysters (*Ostrea edulis* and *Crassostrea gigas*) and clams (*Tapes philippinarum)*. Minimum depuration periods are set.

Spain has many authorized depuration plants with a number relying on gaseous chlorine for disinfection with UV light or ozone systems also in use (Monroy *et al.* 1991). The commercially important species are flat oysters (*Ostrea edulis*), mussels (*Mytilus galloprovincialis*), carpet shells (*Venerupis decussata*, *V. pullastra* and *V. rhomboideus*), striped venus (*Venus gallina*) and the cockle (*Cerastoderma edule*). Minimum depuration periods are set. The systems used are generally large shallow tank arrangements, with the more recent trend towards smaller plants.

In Italy most depuration systems use chlorine disinfection with activated carbon filters being used for dechlorination of the process water. Ozone is also used for the treatment of process water. Most plants use a continuous flow-through of process water rather than closed-circuit recirculation. Some large plants (capacity in excess of 200 tonnes per cycle) are in operation. Minimum depuration periods are set, however these may vary depending on the system design and compliance with bacteriological standards.

The shellfish industry in the Netherlands is centred around the town of Yerseke where mussels (*Mytilus edulis*) and oysters (*Ostrea edulis* and *Crassostrea gigas*) are the main species of commercial interest. The waters in which shellfish are grown are class A and consequently depuration is generally not required except for imported shellfish. However, purification on imported shellfish may be carried out at a small number of plants. Purification times are set according to faecal indicator analysis results on a batch by batch basis.

In England and Wales there are currently about 50 approved purification centres. All systems use recirculation of either artificial or natural seawater with UV disinfection. Seawater generally enters the tank via an operational UV system and is then recycled via the UV unit and the cascade or spray bar to allow aeration. Flow-through systems are not popular primarily due to the difficulty of access to consistently clean and reliable sources of seawater. Re-circulating systems are less vulnerable to pollution incidents. However, dissolved oxygen levels, pH changes and the build-up of inhibitory metabolic by-products (such as ammonia) can present problems if re-use and shellfish/water ratios are not carefully controlled. Four basic designs are currently approved for use: shallow tank, multi-layer system, vertical stack systems, and bulk bin. Minimum depuration criteria are stipulated for all plants as part of the approvals process. In particular, a minimum depuration time of 42 hours is specified for all plants and minimum operating temperatures are specified for each species of shellfish. Other plant specific criteria (such as water flows, operating procedures, etc.) are specified for each plant on a case-by-case basis.

9.4 REMOVAL OF PATHOGENS BY DEPURATION

9.4.1 Control of bacterial infections through depuration

Shellfish-associated typhoid outbreaks were the initial driver for development of commercial shellfish purification procedures early in the last century. Many early studies showed that such systems were effectively able to remove sewage-associated bacteria from shellfish (see section 9.1). Modern depuration systems have been shown on numerous occasions to reduce the levels of bacteria in

sewage contaminated shellfish rapidly and efficiently. Therefore shellfish treated in this manner normally easily comply with bacterial end-product standards. Further evidence for successful removal of bacterial pathogens can be inferred from epidemiological data which shows that common bacterial causes of food poisoning, such as *Salmonella* spp., occur at a surprisingly low incidence in association with consumption of depurated shellfish (West 1985; Anon 1998; Lees 2000). Thus the use of depuration has probably been instrumental in virtually eliminating bacterial illness associated with shellfish consumption in the United Kingdom and other countries where it is extensively used. In the United Kingdom the occasional occurrence of bacterial illness associated with illegally harvested shellfish (not subject to approved commercial treatment processes) reminds us of the continuing presence of bacterial pathogens in sewage contaminated waters and the probable effectiveness of current procedures against them.

9.4.2 Removal of viruses during depuration

Virus removal during depuration is known to be less effective than bacterial removal and a number of outbreaks of viral illness have been associated with the consumption of depurated shellfish (Johnson *et al.* 1990). Epidemiological data suggests that the illness most commonly associated with depurated shellfish is gastroenteritis, principally caused by noroviruses. Noroviruses cause a relatively 'mild' gastroenteritis, often including nausea, diarrhoea, vomiting, fever and abdominal pain. The incubation period is between one and four days with a duration of about two days and generally followed by complete recovery. It is now generally accepted that norovirus is one of the most common causes of infectious intestinal disease in both outbreaks and in the community (Food Standards Agency 2000). Thus sewage can normally be expected to be heavily contaminated with this virus and, indeed, this has been found to be the case (Lodder and Husman 2005). Norovirus has previously been known as Norwalk-like virus (NLV) or as small round structured virus (SRSV). Other gastroenteric viruses, such as astroviruses and parvoviruses, have also occasionally been implicated in shellfish-related outbreaks although their true epidemiological significance is not clear. The other faecal–oral transmitted virus of major significance in shellfish-related outbreaks is HAV (Klontz and Rippey 1991; Conaty *et al.* 2000; Bosch *et al.* 2001).

Compliance of the final product with bacterial faecal indicator standards (such as compliance with <230 *E. coli* per 100 g shellfish) is frequently seen as evidence of satisfactory design and operation of depuration plants. Many risk managers find the simplicity and clarity of such a microbial end-product

standard appealing compared to the effort required for effective scrutiny and approval of complex depuration systems. However, evidence from various sources suggests that depuration plants functioning satisfactorily by faecal indicator criteria may fail to effectively remove human enteric viruses. Perhaps most significant is the epidemiological evidence demonstrating that infection can occur following consumption of depurated shellfish. This was documented in Australia during volunteer trials to assess the safety of depurated shellfish (Grohmann *et al.* 1981) and has also been documented in outbreaks in the United Kingdom, the United States and elsewhere (Murphy 1979; Gill *et al.* 1983; Richards 1985; Heller *et al.* 1986; Chalmers and McMillan 1995; Perrett and Kudesia 1995; Ang 1998). In such outbreaks it is often documented that shellfish are processed in approved commercial depuration facilities in compliance with the requirements of the authorities and that shellfish are also found to be in compliance with the defined bacterial end-product standard. Further data comes from laboratory examination of bivalve molluscs associated with illness outbreaks. Although noroviruses are frequently found, the *E. coli* counts are uniformly compliant with the legal requirements of <230 *E. coli* per 100 g shellfish (Lees *et al.,* unpublished data).

The epidemiological evidence is supported by numerous laboratory studies which have examined elimination rates of human enteric viruses (such as poliovirus) or possible models for the behaviour of human enteric viruses (such as various bacteriophage species), from shellfish during the depuration process (Richards 1988; Power and Collins 1989; Power and Collins 1990; Sobsey and Jaykus 1991; Jaykus *et al.* 1994; Doré and Lees 1995). Although elimination rates in individual studies vary significantly, the overwhelming finding from these studies is that viruses are eliminated from bivalve shellfish at a slower rate than are faecal indicator bacteria (faecal coliforms or *E. coli*). Similar findings are reported for HAV which, unlike norovirus, can be cultured in the laboratory (Abad *et al.* 1997; Demedici *et al.* 2001) and for norovirus using polymerase chain reaction (PCR). Noroviruses were found to efficiently accumulate in shellfish (oysters and clams), but were only poorly removed by depuration compared to *E. coli* (Schwab *et al.* 1998).

In more recent years PCR has been used to study the contamination of molluscan shellfish with noroviruses and HAV at the low concentrations found in field samples. Various studies have shown rather high rates of viral contamination of commercially produced bivalve shellfish placed on the market in a number of different counties (Chironna *et al.* 2002; Formiga-Cruz *et al.* 2002; Cheng *et al.* 2005; Costantini *et al.* 2006). Frequently, these studies found that commercial depuration appeared to have relatively little impact on viral contamination rates and thus that improved procedures were needed to

provide adequate consumer guarantees. Thus, studies performed in commercially marketed shellfish are consistent with the numerous laboratory studies.

The United Kingdom CEFAS laboratory has evaluated virus elimination in commercially depurated shellfish (oysters) as judged by both norovirus, *E. coli* and male specific RNA (FRNA) bacteriophage (a potential viral indicator) content (Dore *et al.* 1998 and unpublished data). Processed shellfish were found to be routinely compliant with faecal coliform end-product standards, however, significant numbers were contaminated with both noroviruses and FRNA bacteriophages. Viral contamination was found to be highly correlated with the degree of harvesting area pollution and to the known incidence of disease outbreaks linked to the site. This data supports previous laboratory findings and confirms that compliance with faecal coliform end-product standards does not provide a guarantee of the absence of enteric viruses in depurated shellfish. A further important finding was that virus contamination, as judged by both norovirus and FRNA bacteriophage content in commercially depurated oysters, was much more prevalent during colder winter months. The dramatic effect of season on viral, but not bacterial, content suggests that physiological requirements for elimination of viruses during shellfish depuration may be significantly different to those required for effective elimination of faecal bacteria. Laboratory studies have suggested that process temperature (Power and Collins 1990; Jaykus *et al.* 1994; Doré *et al.* 1998) may be the most important factor in virus removal during depuration. This is supported by these seasonality findings since the temperature of seawater used in commercial shellfish depuration plants is affected by environmental temperatures. Heaters may be used but generally only to prevent low temperature extremes.

The effect of temperature on virus elimination during depuration requires further study as do other physiologically important parameters such as food availability, salinity, dissolved oxygen, and shellfish condition. Studies in the CEFAS laboratory suggest that a seawater temperature of 18–20°C is optimal for removal of FRNA bacteriophage from oysters (*C. gigas*) but also that successful elimination within a two- to three-day period is critically dependent on initial contamination level (Dore *et al.* 1998). Heavily contaminated shellfish failed to clear bacteriophage within seven days even at elevated temperatures. It is hoped that norovirus removal during depuration can similarly be optimized to maximize the available public health protection. However, a recent molecular study using virus-like particles has suggested that failure to eliminate norovirus during oyster depuration may be due to specific binding of norovirus antigens with specific receptors on the shellfish tissues (Le Guyader *et al.* 2006). If substantiated, this finding suggests that norovirus may prove very difficult to eliminate during depuration, even using optimized procedures.

Until recently, PCR methods for detection of noroviruses and HAV virus were not quantitative. This complicates data interpretation in the virus depuration context since partial removal of virus will not be apparent. The recent advent of quantitative methods for detection of noroviruses and HAV virus in bivalve shellfish using real-time PCR (Jothikumar *et al.* 2005) offers opportunities to further refine our understanding of virus removal during commercial depuration and how to optimize it. In Europe a programme of work has been initiated through the European Committee for Standardisation to produce a standard method for detection of norovirus and HAV virus in foods, including molluscan shellfish, using real-time PCR. The advent of such standardized quantitative procedures for virus detection in molluscan shellfish could revolutionise approaches to both industry and governmental controls for depuration. It is probably the case that many aspects of depuration plant design and operation, optimized to ensure faecal coliform removal, will require re-evaluation in the light of reliable quantitative methods for detection of viruses.

9.5 HACCP FOR DEPURATION SYSTEMS

HACCP analysis is an internationally recognized science-based procedure used to identify and control hazards associated with a food production process. The system focuses on the use of preventive measures rather than end product testing to control risks. By monitoring and controlling each step of the process food processors can ensure that products are as safe as good science and technology can allow. HACCP has been applied to controlling physical, chemical and microbiological risks associated with food and has been widely adopted in the food industry. The process is based on several essential principles:

- conduct a hazard analysis (assess hazards and measures to control them);
- identify critical control points (CCPs) in the production process;
- establish critical limits;
- establish a system to monitor control of CCPs;
- establish corrective action when monitoring indicates CCPs are out of control;
- establish procedures to verify that the HACCP system working effectively;
- establish documentation and traceability for the system.

The application of HACCP procedures to the depuration process has been widely recommended and guidance is available outlining approaches to be adopted for producing HACCP plans for depuration (West 1986; Anon 1999;

Anon 2003; Anon 2006a; Anon 2006b). Although generic guidance is available for introducing HACCP plans it is a critical element of HACCP that plans are specifically tailored to individual depuration centres to ensure the appropriateness of the plan.

In general, HACCP plans applied to depuration have tended to specifically address the removal of bacteria from shellfish rather than virus elimination. This is in recognition of the fact that, in general, depuration as currently practised is not effective at removing viruses from shellfish. Therefore verification of the efficacy of HACCP plans applied to depuration tends to rely on the demonstration of the removal of faecal indicator bacteria to below critical limits established in the HACCP plan. Ideally the critical limits should be more stringent than the minimum regulatory limits to ensure maximal public health protection. Whilst HACCP plans for depuration do not specifically address virus removal it is probably true that the application of a well designed HACCP plan during depuration will increase virus removal (although not always eliminate) when compared with operation where control is exclusively based on simply meeting the regulatory limits for bacterial indicator organisms.

The advantages of applying HACCP to depuration systems is recognized internationally by regulators and is mandatory in several countries. Recently introduced legislation in Europe (Anon 2004a), makes it compulsory for depuration centre operators to implement HACCP. In the United States it is mandatory under the NSSP Model Ordinance (Anon 2003) for shellfish dealers including depuration centre operators to operate HACCP-based controls during the production of shellfish.

9.6 RELAYING

Relaying involves the transfer of harvested animals to cleaner estuaries or inlets for self-purification in the natural environment. This process can be used as an alternative to depuration for lightly polluted shellfish. Shellfish can only be held for relatively short periods in depuration tanks but can obviously be maintained for much longer periods in the natural environment. This makes relaying also suitable for treating more heavily polluted shellfish where longer periods (a minimum of two months is specified in EU Regulations for category C shellfish (Anon 2004c)) are required to remove heavy contaminant loads. The main disadvantages of relaying are the limited availability of suitable un-utilised clean coastal areas and of obtaining ownership rights to those areas, the difficulty of controlling water quality and other water parameters and the

susceptibility of stock to poaching. Combinations of these processes may also be used. For instance in France, traditional practices include holding molluscs in 'claires' (man-made tidally submersible ponds where oysters are held for one or two months to achieve the best quality) and then in 'degorgeoirs' (wet storage ponds) for two days. Relatively little information exists on the removal of viruses during shellfish relaying although again factors such as seawater temperature and initial contamination levels appear critical (Cook and Ellender 1986; Jaykus *et al.* 1994). Data on norovirus and FRNA bacteriophage (Doré *et al.* 1998) and unpublished data suggests that removal of viruses from heavily contaminated shellfish by a combination of relaying for four to six weeks followed by depuration can be effective but again is critically dependant on seawater temperature. In these studies differences were also seen between virus clearance in native (*O. edulis*) and cultured (*C. gigas*) oysters suggesting that species-specific factors should also be considered. Other workers have reported similar data using bacteriophage studies (Humphrey *et al.* 1995). Recent data using molecular methods has provided very similar data for HAV virus (Kingsley and Richards 2003), for rotavirus virus-like particles (Loisy *et al.* 2005) and for norovirus (Le Guyader *et al.* 2003). Thus provision of adequate consumer protection from enteric viruses through relaying of contaminated shellfish in the natural environment is likely to require an extended period of time. Most studies suggest a period in the region of two months depending on water temperature and virus contamination loading.

9.7 CONCLUSIONS

Commercial depuration and relaying procedures are widely used to treat bivalve molluscs harvested from contaminated sites. However, it should be noted that the most effective public health strategy is to focus on good water quality in production areas rather than removal of contamination after the event. Commercial depuration has been shown to be effective for removal of bacterial pathogens but, as currently performed, probably provides only limited health protection against human enteric viruses. The risks are particularly acute for shellfish harvested from heavily contaminated sites. It is thus necessary to monitor the pollution status of bivalve mollusc production areas and permit depuration only from areas compliant with the prescribed standards. The public health protection afforded by commercial depuration can be optimized by making sure shellfish are healthy and metabolically as active as possible and that systems are properly designed and operated. Data on the effectiveness of

depuration for removal of viruses suggests that health protection could be improved by depurating for longer periods and at higher temperatures. Depuration is likely to be ineffective for removal of viruses in unheated systems run for short periods (two days or less) during winter months. An important point is that success in meeting bacterial end product standards can be misleading with regard to the effectiveness of systems for virus removal. It can be dangerous practice to base approval criteria for commercial depuration on simple compliance with bacterial standards rather than on ensuring optimized design and operation of systems. Relaying for extended periods ($>$ two months) is probably effective for removal of viruses from bivalve shellfish except during periods of low seawater temperatures. New quantitative PCR-based detection methods for norovirus and HAV are becoming available for more routine use. These methods will assist our understanding of removal of viruses during commercial depuration and may lead to improved HACCP procedures and public health protection.

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10

Overview of legislative principles and measures

L. Murray and R. Lee

Food safety concerns regarding the consumption of sewage-contaminated bivalve molluscan shellfish, particularly with regard to their role in outbreaks of typhoid, were expressed as far back as the 1890's (Buchan 1910). Large outbreaks of typhoid associated with the consumption of contaminated bivalve mussels and oysters during the early part of the 20th century led to the establishment of national controls in both the United Kingdom and the United States. In England, the Public Health (Shell Fish) Regulations of 1934 allowed local authorities to make orders to control harvesting, or to stipulate further treatment of shellfish (both bivalves and gastropods), from areas deemed to represent a 'danger to public health'. These Regulations, and their equivalents in other Member States of the European Union (EU), were superseded by the implementation of the Shellfish Hygiene Directive (91/492/EEC) which in turn has been replaced by EU Regulation 853/2004, which lays down the specific

hygiene rules for food of animal origin, and by 854/2004 which outlines specific rules for the organization of official controls on products of animal origin intended for human consumption. In the United States, the outbreaks led the Surgeon General to organize in 1925 a conference of relevant federal, state and industry bodies whose recommendations became the basis of the present National Shellfish Sanitation Program (NSSP) (US FDA 2008).

10.1 PRINCIPLE LEGISLATIVE SYSTEMS

The EU and United States systems mentioned above were developed for the purpose of promoting trade as well as for public health controls. In many countries outside these two trading blocks, controls may only be applied to enable export to one, other, or both of these, and produce for local sale may not necessarily be subject to any public health controls. This obviously creates a disparity in public health protection to the detriment of many, especially in less developed countries. In many countries there will be no public health controls on commercial bivalve mollusc production in the absence of any export drivers. In this chapter, reference will principally be made to the EU and the United States. Few countries have any public health controls in relation to the gathering of shellfish for personal consumption, one exception being Canada where such activity is subject to the same controls as commercial harvest (see chapter 12).

Many countries have either signed Memoranda of Understanding with the Food and Drug Administration (FDA) and/or agreements with the EU and have instituted controls that are intended to be equivalent to those of the United States or EU and enable producers in these countries to export to these important markets. The responsible authorities must be recognized as being capable of exerting the appropriate controls and there is the facility for inspection of both the authorities and individual producers. In the case of the EU the responsible authorities within the third country have to recognize individual establishments as complying and a list of these are communicated to the European Commission. The term 'third country' is used to define one which is not within the European Economic Area (EEA); this being comprised of Member States of the EU and certain European Free Trade Association (EFTA) countries. Some countries exporting to both markets have to fulfil the differing requirements of both systems.

The Food and Agricultural Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission implements the Joint FAO/WHO Food Standards Programme which is intended to protect the health of consumers by co-ordinating action by various government and non-governmental bodies

and the preparation of standards and codes of practice. Codes of practice for the hygienic production of a wide range of foodstuffs, including seafoods, have been published by the Commission. The Codex Codes are viewed as the basis for international controls to ensure free trade. Working Groups of the Codex Committee on Fish and Fishery Products have recently produced revised codes of practice for various seafoods including finfish, crustacea and bivalve molluscs (Codex Alimentarius Commission 2009). The revisions to the codes of practice have included incorporation of the principles of hazard analysis and critical control point (HACCP).

The Codex Code of Practice includes an outline of the HACCP process, including a simplified flow diagram for the production of live molluscan shellfish, and sections containing more detail regarding the stages identified on the flow diagram. Each section gives the potential hazards and defects plus technical guidance as to how these might be addressed. Some of the content appears to be more closely related to those in the current EU Hygiene Regulations than those in the US NSSP. This is reflected by a less prescriptive approach to many aspects such as harvesting area controls and depuration requirements.

10.2 INTERACTION WITH OTHER LEGISLATION

In many countries there are interactions between shellfish hygiene legislation and other legislation aimed at controlling pollution. The latter will have the intent of limiting the amount of sewage (and other contamination, such as chemical) to which shellfisheries are exposed which would otherwise limit their utilization due to the requirements of the hygiene legislation. Reducing sewage pollution of shellfisheries at source is one of the most effective ways of reducing the risk of viral illness associated with shellfish consumption.

In the United States, the Federal Water Pollution Control Act (Clean Water Act), as amended, requires that water quality is maintained for the purposes of the protection and propagation of shellfish (Anon 1948). This is undertaken by the Environmental Protection Agency which integrates the shellfish protection duties in with those for other water uses. This results in standards for sewage discharges and diffuse pollution measures. However, in the United States chlorination has been widely used to achieve bacterial indicator standards for sewage discharges and it is known that the viruses of interest with regard to shellfish public health are much more resistant to this compound than are the indicators (Tree *et al*. 1997). Using this approach to achieving compliance could actually increase the public health risk as the classification of harvesting areas will be artificially improved.

In the EU, the codified Shellfish Waters Directive 2006/113/EC (European Communities 2006) is intended to protect shellfish growing waters from pollution, and specifically to "safeguard certain shellfish populations from various harmful consequences resulting from the discharge of pollutant substances into the sea". Most of the requirements of the Directive relate to physical (including temperature and salinity) and chemical parameters which may affect the ability of larval and juvenile shellfish to grow. However, there is a guideline value of 300 faecal coliforms per 100 ml of shellfish flesh and intravalvular fluid in 75% of samples. This is slightly laxer than the class A requirement under the EU hygiene legislation. As the Directive also requires Member States to establish programmes to meet its requirements, this has driven sewage improvement programmes within the EU although a large number of shellfisheries do not meet the faecal coliforms guideline value. Given that the hygiene legislation now concentrates on *E. coli* as a more specific indicator of faecal contamination (see chapter 6), there are often disparities in interpretation of the microbiological status of shellfisheries based on the hygiene requirements and the EU Shellfish Waters Directive. The latter will be subsumed into the Water Framework Directive from 2013 (European Communities 2000).

10.3 GENERAL PRINCIPLES UNDERLYING SHELLFISH HYGIENE CONTROLS

This chapter will address the general principles of legislative controls intended to control public health risks arising from the consumption of bivalve molluscs together with those controls more specifically aimed at the control of the risks associated with microbial contamination of faecal origin. Specific aspects relating to biotoxin, chemical and radiological contaminants will not be addressed; neither will the aspects of some countries' programmes intended to control the risk arising from marine vibrios such as *Vibrio parahaemolyticus* and *V. vulnificus*.

10.3.1 Classification of harvesting areas

Classification is undertaken to provide an assessment of the likely level of contamination by pathogens of the bivalves harvested from the area. The microbiological status of an area is usually based on the results from a monitoring programme using faecal indicator bacteria (see chapter 6). A significant part of the assessment may also involve identification of potential contaminating sources

(from sanitary surveys, see chapter 8). The assessment then dictates whether harvesting will be permitted from an area and, if so, what method and level of treatment may need to be applied to the bivalves prior to further sale.

10.3.2 Mitigation strategies

A primary mitigation strategy applicable to all classes of production area (and also to areas of relaying) is that of the application of short-term controls if the microbiological quality of the harvesting area does not conform to the requirements for the class in question. Such controls may include suspension of harvesting or the application of more severe treatment processes than normally required for the class of area (including relaying instead of depuration – see chapter 9). Suspension of harvesting needs to take into account the fact that faecal indicator bacteria and the pathogens of interest (especially enteric viruses) will clear from the shellfish at different rates and a return to normal status based on the indicators will not guarantee a return to the base level of risk of pathogen contamination. Depending on the degree of contamination, the shellfish species and the seawater temperature, removal of viral pathogens may take from about two weeks to more than two months.

The other mitigation measures involve post-harvesting treatments. The principles of depuration and relaying are detailed in chapter 9. Essentially, these processes are intended to provide the conditions whereby natural functioning of the bivalves will result in purging of microbial contaminants in artificial tanks (depuration) or the natural environment (relaying). Relaying may be undertaken for more extensive periods of time and thus may be used for more contaminated shellfish and, if the time is sufficient, this may result in the removal of enteric viruses. Heat-treatment, under specified conditions, is usually intended for moderately contaminated shellfish and the stipulated conditions should inactivate most non-spore-forming microbes, including enteric viruses. Development of heat-treatment specifications has centred on inactivation of hepatitis A virus, this being more heat resistant than most other pathogens of interest in shellfish-associated illness.

10.3.3 Responsibilities

In general, the hygiene legislation in each country will identify a central competent authority which is responsible for the implementation of the legislation and ensuring appropriate enforcement. Local authorities may be tasked with specific duties such as sampling and practical enforcement. Most legislation in this area puts specific duties on the shellfish industry for ensuring compliance and the

official enforcement duties are principally intended to act as an audit on the industry. In countries with devolved administrations, including states in the United States, regional or local authorities may act directly as the competent authority for regional/local laws. The EU Regulations, effective from 1 January 2006, have direct application in all Member States but there is still the need for these to be applied and enforced on a national, regional and local level. In some cases, individual Member States may introduce national measures in order to apply hygiene rules as allowed for by Community law. In the main these will be used to deal with particular national issues and to enhance public health protection and regulation. In addition, some aspects such as penalties for non-compliance, have to be put into national legislation as the EU does not provide a framework for this.

10.3.4 Traceability

In general, shellfish hygiene control systems incorporate procedures whereby traceability is intended from one stage to the next in the production chain. The means by which this is achieved, and the level of control exerted, varies between the different systems. In general, as for other foodstuffs, both forward and backward traceability (as appropriate) should be possible at any point in the chain. This ensures that if problems are identified in a production or relay area post-harvesting (such as a pollution event), a product can be recalled and if an outbreak of illness occurs, the relevant treatment and packaging premises and production or relay area can be identified for further investigation. In the EU, such documents have to be kept for 90 days in order to allow traceability in the event of an outbreak of illness due to an organism with a prolonged incubation period. Furthermore, these documents have to contain certain prescribed details. In general, commingling of batches should be avoided, if this is allowed in some instances (for example in the EU, batches of the same species and class can be mixed) then full traceability will be lost.

10.3.5 Communication

Effective application of shellfish hygiene controls relies on good communication between the various authorities and between the authorities and the various parts of the industry involved in the production chain. This communication may or may not be specified in legislation: in Europe there is an explicit requirement for food businesses to advise their competent authority where a consignment may present a risk to health. In the case of classified production areas, the central competent authority is required to hold and publish a definitive list and to bring this to the attention of all interested parties. However, effort is required on the part of all

involved to make sure that information is transferred to those who need to receive it. Specifically, the authorities rely on the industry to identify potential new production and relay areas and new depuration or heat treatment plants. The latter are subject to formal processes of approval and so the industry cannot legally trade in the absence of formal application, approval and provision of a unique approval number. Standard application forms are provided for the industry in some member states to facilitate the provision of such information to the relevant authority. The industry also needs to be made aware of any incidents or changes of status in production and relay areas or of potential outbreaks of shellfish-associated illness.

10.3.6 Funding

In general, funding for the official shellfish hygiene controls is provided by central and local authorities while the industry is expected to fund those aspects of control for which it is responsible (such as end-product testing). In some systems the industry is also expected to make a contribution to, or largely cover the costs of, the official controls. The new EU Food Hygiene Regulations (see below), make allowance for the competent authority to make a charge on industry for the funding of the monitoring programme and for 'excessive' official controls relating to the expenses incurred during control of large incidents. However, in general there are usually significant hidden costs of the official controls that fall to public funding.

10.4 EU LEGISLATION

Current EU legislation on live bivalve molluscs (which also covers gastropods, echinoderms and tunicates) are included in hygiene legislation concerned with all food of animal origin. Regulation (EC) No 853/2004 (European Communities 2004a) covers the hygiene rules to be applied by harvesters/businesses and Regulation (EC) No 854/2004 (European Communities 2004b) those for official controls to be applied by the competent authority. The controls only apply to commercial production. This legislation replaces controls which were previously applied under Directive 91/492/EEC, the Shellfish Hygiene Directive, as implemented in national legislation in each Member State. Casual gathering of shellfish for home consumption is not subject to any EU hygiene controls. Member States are responsible for passing their own legislation implementing the Regulations. The UK national legislation implementing the Regulations was put in place in January 2006. Details of the implementing legislation may vary between the Member States and in some instances these variations have caused significant differences in the form and extent of controls. In some areas the Regulation does not specify requirements in detail and this will increase the opportunity for

variation in interpretation of the requirements between Member States. Where any differences or local specifications apply, these will be highlighted in the appropriate sections. Reference will also be made where appropriate to potential significant differences in application in other Member States.

Control within each Member State is the responsibility of the competent authority; the body or bodies which undertakes veterinary checks. In the United Kingdom the Food Standards Agency (FSA) has competency for the monitoring of harvesting areas and the enforcement of these at central level. Local enforcement is the responsibility of the local food authority which consists of local authorities and port health authorities. These also undertake the official control sampling of harvesting areas on behalf of central government. In the United Kingdom both the Official Controls Regulation 854/2004 and the hygiene rules Regulation 853/2004 are to be implemented by a single set of Regulations.

Shellfish imported into the EU from a third country must have been produced under conditions which are at least equivalent to those stipulated by the Regulations. Countries outside the European Economic Area may apply for equivalence under which they can trade with the EU on the same basis as Member States. The Commission will then verify the controls applied by the third country to fulfil the relevant requirements and will publish a list of those centres within the country that are deemed to satisfy the controls. Where health problems and/or the presence of pathogens have been identified in third country imports, the Commission may impose particular controls until it is satisfied that any deficiencies have been rectified.

The following sections will concentrate on the requirements of the Shellfish Hygiene specific Annexes of Regulations 853/2004 and 854/2004. Regulation 854/2004 Annex II applies to live bivalve molluscs and by analogy to live echinoderms, live tunicates and live marine gastropods. It stipulates controls at all stages of production from monitoring of the quality of harvesting areas through to placing on the market. A large proportion of the content of the Regulations, as they relate to shellfish, is directed at addressing the health problems caused by sewage contamination.

Where shellfish are to be further processed by cooking, canning, or other means, they must meet the requirements of live bivalve molluscs prior to such processing. The controls under the Regulations stipulate:

- classification of production and relaying areas;
- monitoring of classified production areas and relaying areas to determine the degree of faecal pollution according to defined *E. coli* parameters, which results in the designation of each production area to one of three classes;

- decisions after monitoring;
- additional monitoring requirements;
- recording and exchange of information; and
- food business operators' own checks.

10.4.1 Classification of production and relaying areas

Prior to classification, there is a requirement to undertake a study to determine the sources of organic pollutants, the way that they vary with season and the way that contaminating effects are modified by the bathymetry and hydrodynamics within an area. This essentially constitutes a sanitary survey. Classification itself then requires demonstration of compliance with criteria for *E. coli* in shellfish flesh. The levels are given in chapter 6. These determine whether areas are classified as A, B or C. Shellfish harvested from class A areas can be sold for human consumption without further treatment. Those from class B areas must be depurated or relayed in class A areas and those from class C areas must be relayed for an extended period of time (up to two months) or subjected to an approved heat-treatment procedure.

10.4.2 Monitoring of production and relay areas

These areas have to be periodically monitored for microbiological quality, toxin producing plankton, biotoxins and chemical contaminants. There is also a need to check the origin and destination of bivalve molluscs from these areas. Sampling plans have to be prepared for this monitoring and these have to ensure that the monitoring is both representative and reflects geographical and temporal variation. The default sampling frequency for biotoxins is defined as weekly during periods of active harvesting, unless a risk analysis shows that a reduced frequency is justified.

10.4.3 Decisions after monitoring

If monitoring shows that the relevant standards are exceeded, or even if not, there might be a risk to human health, in which circumstance the production area has to be closed to prevent harvesting. An alternative is to re-classify the area appropriately. The methods for this are explained in chapter 11.

10.4.4 Additional monitoring requirements

Any classified production area that is closed or subject to special conditions in relation to harvesting has to be policed in order to ensure that bivalves from such

areas do not end up on the market. There is also a requirement for verification checks to be made at the end-product stage. This is in addition to the requirements for testing by the food business operators themselves.

10.4.5 Recording and exchange of information

The competent authority has to maintain a list of classified production and relay areas and to provide this to interested parties such as those involved in the bivalve mollusc trade. Those interested parties have to be informed when there is a change in the hygiene status or extent of a production area.

10.4.6 Food business operators own checks

Food business operators are required to undertake their own checks in depuration and dispatch centres. Results of these internal checks may be taken into account in determining the classification of production areas, and in any decision to close or open such areas, providing that the sampling and analysis have taken place in accordance with agreed protocols, and that the laboratory has been designated by the authority and is accredited to acceptable standards.

10.4.7 Controls on harvesting, storage and transport

10.4.7.1 Harvesting

Shellfish must be harvested from areas that conform to the criteria for class A, B or C as determined by the microbiological monitoring described above and then subjected to any appropriate processing dictated by the classification. The competent authorities may prohibit the harvesting on health grounds from areas that meet the specified monitoring requirements. Temporary closures may be undertaken where particular contamination events have occurred; in the United Kingdom these are known as Temporary Closure Notices (TCNs) and are made by the local food authority. In general, in the United Kingdom such TCNs are used for sewage contamination, such as in the case of emergency discharges arising from equipment breakdown at a sewage plant.

Compliance with the geographical limits of classified harvesting areas and the correct identification of the origin of batches (see section 10.4.11 on Documentation) rely greatly on the co-operation of harvesters with some auditing by the authorities. In the United Kingdom, the food authorities are often aided in such matters by other bodies with responsibility for controls on shellfish stock conservation, the latter more often having access to suitable boats and

other equipment. Verification of practices in mariculture areas tends to be easier than that of the harvesting of wild stocks.

10.4.7.2 Storage and transport

These activities may take place both before and after processing and/or packing: if processing is by heat treatment, smoking, or similar means, then the requirements of the Hygiene Regulations relating to Fishery Products will apply. General hygiene controls apply to avoid contamination. There are specific stipulations precluding immersion in more contaminated water after harvesting or any form of immersion after leaving the dispatch centre (see below), both of which could negate the effects of other controls. The comparable controls in the United States include specification of storage and transport temperatures in order to limit the multiplication of bacterial pathogens. No such specifications are given in the European Regulations.

10.4.8 Standards for depuration, relaying and heat treatment

10.4.8.1 Depuration

Purification centres have to be approved by the competent authority. The regulations contain a mixture of general and specific requirements given regarding purification systems and processes. The general nature of much of the requirements has led to wide variations between EU Member States regarding depuration system requirements and practices. The requirements are also less stringent than those which applied in many European countries under domestic legislation preceding the Shellfish Hygiene Directive (which applied before the EU Hygiene Regulations), for example in Denmark and France.

   This particularly applies to the period of purification. No particular period is specified in Regulation 853/2004. It is, however, stated that the period must be sufficient for the shellfish to meet the microbiological end-product standards and that it should be adjusted, where necessary, to meet the extent of contamination of the incoming product. Historically, many EU Member States had standard stipulated minimum depuration periods, usually in the region of 48 hours, and some removed the standard requirement when the Shellfish Hygiene Directive (91/492/EEC) was introduced. This, together with broad interpretations of the other requirements in ways that do not conform to good practice based on best current technical knowledge, has led to the situation in some Member States where purification of class B shellfish may fail to produce shellfish that will consistently meet the *E. coli* and salmonella end-product standards. In the United Kingdom, a standard 42-hour depuration period has been maintained and

conformance with this and other prescribed operating conditions results in virtually all post-purification testing showing compliance with the bacterial end-product standards. In Italy, a 48-hour purification period is stipulated for shellfish imported from third countries (Italian Republic, 1993).

The frequency and type of microbiological testing of shellfish before and after purification and any testing of the seawater used for depuration will be determined by the operator's HACCP plan. The final product will need to meet the standards described below in section 10.4.10.

10.4.8.2 Relaying

For class C shellfish relaying is an alternative to heat treatment and for class B shellfish an alternative to depuration. Where class C shellfish are relayed in class B areas they will still require depuration after the necessary relay period. Specific stipulations are given in Regulation 853/2004 regarding the identification of relay areas, their separation from production areas, and the operation of a batch system, in order to enhance control of the process. The competent authorities can determine the minimum water temperature which is deemed necessary for effective removal of contaminants. As with purification, the period required is specified by the criteria of the faecal indicator bacteria. There is an additional requirement for class C shellfish to be relayed for at least two months irrespective of this, although the competent authority can agree to a shorter period if a risk assessment shows this to be justified. This is another aspect of the application of the EU hygiene controls where there has been a difference in implementation between Member States: in some, relay of class C shellfish may take place in class A or B waters (with subsequent depuration in the latter case), whereas in others relaying is only permitted in class A waters.

10.4.8.3 Heat treatment

Approved heat-treatment methods are recognized alternatives to relaying for class C shellfish and depuration for class B shellfish. The methods are specified in Regulation 854/2004 as:

(1) immersion in boiling water for the period required to raise the internal temperature of the mollusc flesh to not less than 90°C and maintenance of this minimum temperature for a period of not less than 90 seconds;

(2) cooking for three to five minutes in an enclosed space where the temperature is between 120 and 160°C and the pressure is between 2 and 5 kg/cm^2, followed by shelling and freezing of the flesh to a core temperature of –20°C; and

(3) steaming under pressure in an enclosed space satisfying the requirements relating to cooking time and the internal temperature of the mollusc flesh mentioned under (1). A validated methodology must be used. Procedures based on the HACCP principles must be in place to verify the uniform distribution of heat.

10.4.9 General hygiene standards

These controls are prescribed to ensure that transport facilities and buildings and equipment used in purification and dispatch centres do not contribute to contamination of shellfish and are readily cleanable, thus reducing such risks further. Dispatch centres are facilities undertaking final washing, grading and wrapping of products ready for human consumption. Such centres must also be approved by the competent authority.

10.4.10 End product standards

Live bivalve molluscs sold in the final state for human consumption have to meet the following requirements:

(1) they must have organoleptic characteristics associated with freshness and viability, including shells free of dirt, an adequate response to percussion and normal amounts of intravalvular liquid;
(2) they must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately) that exceed the following limits:
   (a) for paralytic shellfish poison, 800 µg/kg;
   (b) for amnesic shellfish poison, 20 mg domoic acid/kg;
   (c) for okadaic acid, dinophysistoxins and pectenotoxins together, 160 µg okadaic acid equivalents/kg;
   (d) for yessotoxins, 1 mg yessotoxin equivalent/ kg; and
   (e) for azaspiracids, 160 µg azaspiracid equivalents/ kg.

   EU Regulation 2074/2005
   (1) *E. coli* ≤230/100 g;
   (2) *Salmonella* spp. not detected in 25 g.

It is recognized in the Regulations that scientific progress may result in the application of tests for viruses rather than relying only on faecal indicator bacteria as a guide to freedom from microbial contaminants. Occurrences of

large viral outbreaks ensuing from intra-community trade in live oysters
prompted a review of documentation requirements (see below) and the
establishment of a network of European and National Reference Laboratories
for the microbiological aspects of shellfish hygiene.

10.4.11 Documentation ("paper trail")

The documentation specified in Regulation 853/2004 is intended to provide a
means whereby the origin of shellfish and any subsequent processing can be
determined in the case of illness associated with shellfish. This provides a means
whereby the responsible authorities can undertake appropriate checks of the
harvesting area, approved centres and other factors which have been involved
with such a batch of shellfish.

Each harvested batch of shellfish destined for a purification centre, dispatch
centre or fishery products establishment must be accompanied during transport
by a registration document containing a number of items of information
regarding the harvester, the date and place of harvesting, the species and
approximate quantity, and the approval number and destination of the batch. The
responsibility for completing these documents lies with the harvester.

In order to improve traceability, required as a result of problems encountered
during investigation of intra-community outbreaks, the registration document
requirements were amended and the document must now contain details of the
health status of the production area. The registration documents for relayed
shellfish must also include information on the length of relay. Where a
registration document is to accompany a batch of shellfish from a purification
centre to a separate dispatch centre, the duration of purification must be
recorded.

In some EU Member States, there is a requirement for fishermen to keep
record in a logbook of the co-ordinates, place and class of harvested shellfish as
well as the appropriate information being included on the registration document.
This provides an additional check on the information given on the movement
document.

Packages of shellfish leaving a dispatch centre (or combined purification/
dispatch centre) must be provided with a health mark identifying factors
including the dispatch centre (by means of a unique number), country of
dispatch, species, day and month of wrapping. The health mark must also
include either a warning that the shellfish must be alive when sold or a date of
durability. Where the shellfish are not in individual consumer-sized packages,
the health marks must be retained by the retailer for at least 60 days following
the "splitting" of a consignment.

The connection between the health-marked shellfish and the movement documentation showing harvesting details should be provided by the documentation within the purification or dispatch centre. The establishment of such a connection may be complicated by the allowance of the mixing of batches from different areas of the same health status: traceability in such situations will essentially stop at the purification/dispatch centre.

10.4.12 Imports from third countries

The requirements for equivalence were referred to in general terms in section 10.1. These are explicitly defined in the EU 853/2004. A recent list of countries deemed equivalent under Community law have been published by the Commission (European Communities, 1997a, amended by European Communities, 1997b). The equivalence requirements under the EU Shellfish Hygiene Directive also apply to imported processed shellfish that must meet the requirements of that Directive prior to processing (i.e. bivalves, echinoderms, tunicates and marine gastropods).

10.5 UNITED STATES NATIONAL SHELLFISH SANITATION PROGRAMME

10.5.1 United States legislation

United States controls are exerted at the federal and state levels. The FDA is the prime federal agency responsible for regulating seafood safety. The power for such regulations derives from the Federal Food, Drug and Cosmetic Act (US FDA 1989) and the Public Health Service Act (US PHSA 1944) and these control interstate trade. The National Marine Fisheries Service of the Department of Commerce undertakes a Voluntary Seafood Inspection Programme. Other federal agencies are also involved in seafood safety programmes. State authorities are responsible for intra-state controls and play a significant part in the co-operative National Shellfish Sanitation Programme (NSSP).

The NSSP exerts voluntary controls on the interstate trade in bivalve molluscs. The FDA has a key role in the administration of the programme, formulating regulations and overseeing state controls. State agencies are responsible for passing and implementing state laws and regulations consistent with the National Programme. The state agencies are then responsible for applying these controls within their own states, with the FDA undertaking audits of their effectiveness of compliance with the programme. Industry co-operates

by ensuring that shellfish are only accepted from recognized sources. The Interstate Shellfish Sanitation Conference consists of officials from federal and state agencies and industry and reviews the performance of the programme. The FDA produces a manual, subject to periodic revision, which specifies the criteria for compliance with the programme (US FDA 2008). The FDA also uses these criteria to establish Memoranda of Understanding with other countries wishing to export shellfish to the United States.

10.5.2 Classification of harvesting areas

Controls on the microbiological quality of harvesting areas are undertaken via the testing of water rather than of shellfish as in the EU Directive. Thus all species occurring within a single area will have the same classification. There are effectively five classes of waters (see also chapter 6). Shellfish from approved waters can be sold directly on the market without prior treatment. Shellfish from restricted areas may only be sold after depuration or relaying. These two classes therefore correspond to class A and B respectively of the European Shellfish Hygiene Directive. Conditionally approved or conditionally restricted areas are known to be subject to periodic pollution and during, and for a period of time after such events, particularly periods of heavy rainfall, the area will be closed. Shellfish from prohibited areas may not be sold for consumption. Any class of area may be subject to closure by the state authorities if pollution dictates. Within harvesting areas prohibited areas are defined around sewage outfalls or other sources of contamination. Separate control criteria are given for marinas that may be subject to particular types of intermittent pollution.

Classification of harvesting areas depends on a shoreline survey of the area to identify potential sources of pollution, an analysis of meteorological, hydrographic and geographic factors affecting the area and bacteriological analysis of water samples. This information has to be updated annually and re-evaluated every three years. A full survey has to be undertaken for each area at least every 12 years. Minimum numbers of water samples are prescribed for each of these evaluations, a number of which have to be taken under conditions which are determined to yield the worst results. Water samples are either tested by an MPN method which is specified by the American Public Health Association (APHA 1970) or by a membrane filtration method.

If shellfish-associated illnesses are linked to a particular harvesting area, or if pathogenic organisms are isolated from shellfish samples from a harvesting area, then the classification of the area has to be reviewed and, if necessary, amended. The other essential components of the NSSP also have to be reviewed for possible deficiencies.

The NSSP gives state authorities the option of clearing adult and developing shellfish from prohibited areas in order to prevent illegal harvesting. Seed shellfish, as defined by the authority, which are taken from a prohibited area must be grown on in an aquaculture area for at least six months prior to harvesting for human consumption.

10.5.3 Harvesting and transport

General hygiene rules are stipulated for the harvesting process to prevent contamination of product. Harvesters have to be licensed by the responsible authority and such authorities are responsible for undertaking patrols of the various categories of harvesting areas.

Time limits are specified by which shellfish not intended for wet storage or depuration must be placed under temperature control. These time limits are intended to prevent proliferation of bacterial pathogens. More stringent temperature controls apply if the waters have been associated with *V. vulnificus* infections.

Stipulations are given that boats involved in the shellfish trade (harvesting or others) should not dispose of sewage into harvest areas. Failure to comply with this requirement resulted in a large outbreak of viral illness (Kohn *et al*. 1995).

10.5.4 Holding, shucking, heat shocking and packing

General hygiene stipulations are given for these operations together with requirements for the quality of water used for wet storage and control of temperatures during processing and, packing and storage.

10.5.5 Depuration

Plans for plants have to be approved by the authority before construction. Specifications regarding the location and construction of plants and equipment are given and also for the seawater used (dissolved oxygen, coliforms, salinity, temperature, pH), sorting and cleaning of shellfish prior to depuration, loading of shellfish (tank/trays) and flow of water. The minimum depuration period allowed is 48 hours.

A control plan for the plant has to be prepared by the authority and plants have to be inspected and certified prior to operation and at intervals thereafter, the plants have to have a satisfactory supervisory system and have to keep records regarding both the source shellfish and the depuration process. The process to be undertaken has to be specified and approved and tested to

demonstrate compliance with the recommended removal efficiency for removal of faecal coliforms. Ongoing verification criteria for depuration systems (based on analysis of results from ten process batches) are given in Table 10.1. Where full verification has not been achieved for a plant or new source of shellfish, or where failure of the criteria has occurred, the shellfish post-depuration must meet the following criteria:

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

Table 10.1 United States NSSP criteria for verification of depuration plant performance

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

Source: US FDA, 2008

10.5.6 Documentation

All stages of the production of shellfish, from harvest to final packing have strict requirements for appropriate marking of batches and identification of the harvester, processing establishment, and all other components through which the shellfish have passed. Separation and identification of independently harvested batches has to be maintained through the production system, unless accommodated by a management plan which minimizes such mixing and specifies how identification of source will be maintained. The information is cumulative and does not apply to separate stages of the harvesting and processing chain. This would seem to have advantages over the EU system in which it may be difficult to make adequate links between the separate items of documentation involved in the production process.

10.5.7 Control of laboratories

Laboratories taking part in the bacteriological examination of water samples for classification purposes and shellfish samples for end-product testing are inspected under the control of the FDA. The FDA also undertakes distribution of split samples to such laboratories under the Shellfish Laboratory Quality Assurance Program (Peeler *et al.* 1995).

10.6 CONTROLS OUTSIDE THE EU AND UNITED STATES

Those countries that export to one or other of the EU and United States will need to satisfy the relevant requirements of the importing bloc, this being either direct application of the controls which apply in the EU or United States, or which are recognized as being equivalent to these. There is usually a need for the competent authority in the exporting country to show that it has the ability to apply and enforcement the requirements.

In countries such as Canada and New Zealand that export to both the EU and United States, the regulatory and enforcement system will need to be acceptable to both. In practice, rather than applying two different systems, such countries have tended to develop modifications of one or other, or hybrids of the two, that are not only acceptable to the importing authorities but may have unique features and advantages. Further details on the systems applied in Canada and New Zealand are given in chapters 12 and 13. In these countries, the hygiene controls are also applied to products for domestic consumption but this is not necessarily the case. In many countries that do not export to the EU or United States, there are no hygiene controls on commercial shellfish production.

10.7 CONCLUSIONS

Due to the large amount of international trade in shellfish, many countries have controls based on either the EU or United States standards for hygiene of seafoods and some may have to satisfy both. Addressing the problems of the inconsistencies between the two systems may be progressed via a body such as Codex Alimentarius, but such consolidations need to be undertaken without "generalisations" which may dilute the effectiveness of each system and may also lead to inconsistencies in the interpretation and application of the controls in different countries. The EU and United States hygiene requirements for imports of shellfish may also lead to significantly different standards for shellfish

exported from a number of developing countries and those that are applied to shellfish produced for consumption within those countries.

Ultimately, public health legislation, and its enforcement, needs to be judged on its effectiveness in controlling and preferably reducing the incidence of infection associated with the products in question. This requires the acquisition of good epidemiological data and a mechanism for ongoing scrutiny of such data. Evaluation of information from individual outbreaks can yield valuable additional information regarding any possible causes and enable the identification of possible improvements in legislation and/or enforcement. Reduction in the incidence of viral illnesses associated with consumption of bivalve molluscs will necessitate improvements in technology, both laboratory and processing, as well as the appropriate use of legislation.

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11

Official control monitoring programmes for live bivalve molluscs – legislative and regulatory approaches: Scotland

L. Murray

This chapter should be read in conjunction with chapters 10, 12, 13 and 14. Together these five chapters provide a detailed overview of existing legislation in a number of key countries whilst also highlighting the gaps, anomalies and principal issues that face those charged with the responsibility for monitoring and control programmes for live bivalve shellfish and their waters.

11.1 BACKGROUND AND CENTRAL COMPETENT AUTHORITY ROLE IN SHELLFISH CONTROLS – THE FOOD STANDARDS AGENCY

The Food Standards Agency (FSA; The Agency) in the United Kingdom is an independent food safety watchdog set up by an Act of Parliament in April 2000 to protect the public's health and consumer interests in relation to food. Although the FSA is a Government agency, it works at "arm's length" from Government because it does not report to a specific minister and is free to publish any advice it issues. The Agency is accountable to Parliament through Health Ministers and to the devolved administrations in Scotland, Wales and Northern Ireland for its activities within their areas.

The Food Standards Agency Scotland (FSAS) is the central competent authority under European Union (EU) Regulation 854/2004 (European Communities 2004a) and in this capacity is responsible for implementing official controls in relation to live bivalve molluscs. This Regulation is directly applicable in its entirety throughout all European states. Local enforcement powers are delegated in Scotland through the Food Hygiene (Scotland) Regulations 2006.

The Agency undertakes official programmes in relation to microbiological assessment of live bivalve mollusc production areas, administers and classifies all live bivalve mollusc production areas, oversees the approval of depuration facilities and also undertakes the monitoring of each area for biotoxins, phytoplankton and chemical contaminants. The components of the classification system and associated microbiological components will be discussed within this chapter.

11.1.1 Overview of programme

Scotland currently has 186 classified shellfish harvesting production areas covering 246 individual harvesting sites. Six main species of bivalve mollusc are classified. The majority of the production areas are found in the Shetland Isles, Argyll and Bute, and across the Highland areas of Lochaber, Skye and Lochalsh, Ross and Cromarty, Sutherland and Inverness. Further classified production areas are found in Orkney, Western Isles, North Ayrshire, Edinburgh, Moray, Dumfries and Galloway and Fife. The latter areas tend to be home to wild shellfisheries rather than aquaculture sites and hold species such as clam species and common cockles. Within aquaculture areas, common mussel is the main species with Pacific Oyster also being a major species, particularly in the Argyll and Bute area of Scotland. Figure 11.1 shows the location of main shellfish production areas.
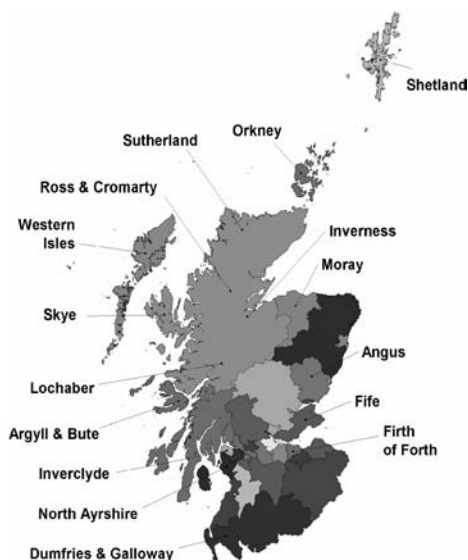
Figure 11.1. Scottish areas where shellfish are provided for the analysis of *E. coli* for the harvesting areas to be classified.

11.2 MICROBIOLOGICAL OFFICIAL CONTROL MONITORING PROGRAMMES MANAGED BY THE FOOD STANDARDS AGENCY SCOTLAND

In accordance with Annex II of the EU Hygiene Regulation 854/2004 (European Communities 2004), the FSAS is required to establish the location and fix the boundaries of shellfish harvesting areas. The process involved in area classification is stringent and includes regular sampling of shellfish from representative monitoring points by Enforcement Officers from each Local Authority area, with the assistance of shellfish harvesters. Twelve local food authorities (LFA) are involved in the programme. The regulations stipulate that the competent authority must monitor the levels of *E. coli* within the harvesting area and that according to the sample results, must classify the area as being one of three categories; A, B or C. An A classification allows for the product to be placed directly on the market, whereas B or C classification requires the product to go through a process of depuration (purification), approved heat treatment or relaying before it can be placed on the market (see chapter 9).

Regulation (EC) No 854/2004 (European Communities 2004a) states that if the competent authority decides in principle to classify a production or relaying area, it must:

(1) make an inventory of the sources of pollution of human or animal origin likely to be a source of contamination for the production area;
(2) examine the quantities of organic pollutants which are released during the different periods of the year, according to the seasonal variations of both human and animal populations in the catchment area, rainfall readings, wastewater treatment and other similar factors;
(3) determine the characteristics of the circulation of pollutants by virtue of current patterns, bathymetry and the tidal cycle in the production area; and
(4) establish a sampling programme of bivalve molluscs in the production area which is based on the examination of established data, and with a number of samples, a geographical distribution of the sampling points and a sampling frequency which must ensure that the results of the analysis are as representative as possible for the area considered (European Communities 2004a).

In essence, the requirements given in paragraphs (a–c) of the Regulation constitute what has been termed a sanitary survey and paragraph (d) shows that the contents of this should influence the content of the sampling plan.

The Regulations also require the competent authority to undertake sanitary surveys for all new areas. It is additionally the intention of the FSAS to undertake such surveys on all of their existing classified production areas over a reasonable period of time. A formal report will be produced for each area, containing the outcome of the various elements together with an overall assessment of the effects on the shellfishery. The survey information will be reviewed on a regular basis and the LFAs will contribute information to this review. The final product will be the sampling plan(s) for the area. The information contained in these plans will include:

• production area;
• site name and identification;
• geographical location;
• depth of sampling (if relevant);
• frequency of sampling;
• responsible authority; and
• authorised sampler(s).

Currently the FSAS carries out its annual review of classifications every December and awards provisional, seasonal or full classifications on the basis of three years historical *E. coli* monitoring data. A minimum of nine samples is required, taken in separate months between January and December, to maintain an area classification. However, if the shellfish harvesting area is a new site and is yet to be classified or has a history of fluctuating results during specific months, then the minimum sampling frequency will be recommended to be more frequent. A separate fast track classification system also exists, which allows for the immediate harvesting of previously unclassified or a declassified area for a single season in that year. FSAS officers award a provisional B classification for this harvesting period after a general desktop survey has taken place.

In addition to monitoring of microbiological criteria within these classified shellfish production area, programmes for the monitoring of toxic species of phytoplankton and biotoxins specified in EU854/2004 (European Communities 2004a) are also undertaken. These programmes are not discussed further under the remit of this chapter.

11.2.1 Classification of new shellfish production areas

New shellfish production areas may be classified at any point during the year when the minimum number of samples has been submitted and results received. A properly completed classification form is essential before the FSAS will proceed with the classification. A standard form is supplied and requires data on sampling locations and full historic details of the area. It also requires full details of the harvester, intended site harvesting patterns and LFA details. FSAS undertake a site assessment prior to classifying a new area. This involves liaison with other government agencies, the LFA and the harvester as necessary. This allows the FSA to award classification in full consideration of available information. The classification decision is communicated to the harvester, LFA and relevant laboratories contracted to carry out official control sample analysis. The awarding letter stipulates a unique reference number for the area which must be used on all sample submission forms and labels. In addition, a standard grid reference from which samples must be taken is stated. The area boundaries are set and any limitations to the classification stated. An opportunity is also taken to reinforce to the harvester their legal responsibilities.

A production area may be given a provisional classification status where the site results are variable. This area will then be subject to continuous review to allow alteration of the classification if required. Once an established trend from results is apparent (a minimum of three years consistent data is required), an area may be given a season classification which splits the classification category over

the year dependent on the pattern observed. An area with proven stable results will be given a full and permanent classification status for the year.

Harvesters are able to appeal against the classification award and an appeals panel comprising FSAS staff and other experts consider these appeals. The FSAS publish a yearly classification document which includes a full protocol on classification and appeals, it also provides classification and appeal application forms.

11.3 RESPONSIBILITIES AND INTERACTION WITH OTHER AGENCIES

11.3.1 Interaction with the Local Food Authority

The FSA delegates competence for certain official controls to the LFA. Therefore, enforcement of the provisions is carried out by a combination of central and local government, with The Agency retaining overall accountability to the European Commission (EC).

Within Scotland there are 32 LFAs, around half of these have direct and indirect involvement in the controls required in relation to live bivalve molluscs. This may be through the local management of production areas, approval and inspection of depuration facilities, inspection at first landings and auction markets or at the processor. These arrangements necessitate close working between the central and local authorities and between the LFAs themselves. This has been facilitated by formal liaison arrangements made under a joint enforcement working group the Scottish Fish Hygiene Working Group. This group contains representatives of the FSAS and all LFAs with a shellfish interest. In addition, The Agency provides guidance and direction to LFAs via formal statutory Enforcement Codes of Practice and Guidance. This is seen as a mandatory activity by the Food and Veterinary Office of the EU.

The FSA directly engage with the LFA Officers throughout the classification process and in relation to the monitoring programmes as a whole. The Scottish Fish Hygiene Working Group, meets four times a year. Additional focussed meetings may be called on a more regular basis where necessary. The group are consulted on FSA guidance in the relation to shellfish and assist in identifying officer training needs in the area. These needs are supported by the FSA under a low cost training initiative. This initiative financially assists LFAs in staff training.

The LFAs are audited by the FSAS to ensure that they are implementing the legislation properly. The FSAS as central competent authority is subject to audit by the Food and Veterinary Officers of the EC to ensure that it is properly and adequately implementing the relevant European Directives and associated decisions.

11.3.2 Interaction with consumers

The FSAS has the principle of consumer protection as one of its core values. Interaction with consumers is therefore an important aspect of the FSA's work. All production area classifications are communicated to all interested parties and additionally published on the FSA website.

This allows consumers direct access to classification details. In addition, the United Kingdom legislation ensures the clear labelling of all shellfish. This ensures that the consumer has knowledge as to source and classification of shellfish purchased in a retail establishment in a wrapped condition.

Any public shellfish gathering area is subject to public notices and press publicity if any harvesting restrictions apply. This ensures that casual gatherers are alerted to the potential danger of gathering shellfish from the contaminated area.

Furthermore, any allegations of foodborne illness as a result of shellfish consumption is subject to formal investigation through a network of public health specialists including the LFAs and Community Public Health Medicine Specialists.

11.3.3 Interaction with industry

The microbiological official control monitoring programme results in the collation of data which allows the classification of the shellfish harvesting areas to be determined. This programme is therefore of direct interest to industry whose business is partly dependent upon the classification granted by the FSA.

Industry is advised on an ongoing basis of all results pertinent to their site. This information is communicated via the LFA who is provided with all site results by the FSA on a weekly basis. Any results found to be outside the current classification status are communicated immediately to allow the harvester and LFA to conduct an on-site investigation and re-sampled where determined necessary. The harvester is essential to the proper running of the monitoring programme. They assist the LFA in collecting and transporting samples to the contract laboratory. As such they have an integral role and interest in the programme. In order to support their role the FSA has produced guidance and protocols in the advised method of sample collection and transportation as well as protocols on the classification system as a whole.

It is imperative within Scotland to have a system which allows classification decisions which have such importance to the individual harvesters to be open to challenge and appeal. As a result the FSA has a formal appeals procedure which allows harvesters to appeal their annual classification. The basis for appeal must

be communicated in writing using a standard application form. During this process the harvester may ask for the results of his own sample analysis to be accounted. It is mandatory that the samples have been taken by approved methods and analysed using the National Reference Laboratory accredited method in a United Kingdom Accreditation Service approved laboratory.

The FSA meets regularly with the main industry body, the Association of Scottish Shellfish Gatherers. This assists in ensuring adequate communication and also allows the FSA to ensure that industry responsibilities are properly understood. EU Regulation places responsibility upon the industry to ensure that shellfish placed on the market are within their given classification and meet end product standards. Compliance with these requirements is audited and enforced by the LFA.

11.3.4 Interaction with other official bodies

The Scottish Environmental Protection Agency (SEPA) has a key role in the monitoring of designated waters and in the control of pollution within these areas. The FSA promotes communication with SEPA where microbiological results are received which indicate that a pollution event may have occurred within a classified shellfish production area. The LFA is responsible for local communication with local Scottish Environmental Protection Agency officers. Local action groups, which comprise officers from SEPA, the LFA, Scottish Water and industry will investigate any unusual events with an objective to ensure that there is no ongoing contamination event which may affect shellfish safety.

On a regular basis the outcome of full sanitary surveys are shared with these bodies to assist in determining risks and action strategies to limit impact upon local shellfisheries.

11.4 MITIGATION MEASURES

11.4.1 Action where samples results are outside the classification limit

All samples received by the laboratory which are outside the current area limit are reported immediately. This information is directed to the LFA who communicate with the harvester as necessary. Dependant on circumstances (such as no natural explanation for elevated result, failure of repeat result), a site visit for investigation purposes may be undertaken as determined by a local action group. A resample may be taken and the harvester's end-product test results will be inspected. This investigation may result in the classification

status being altered, depuration being instigated or in emergency situations, a Temporary Closure Notice being administered as allowed under EU 854/2004 (European Communities 2004). On these occasions communication with other bodies as described under section 11.3.4 takes place.

11.4.2 Treatment measures

In recognition of the fact that the process of depuration of shellfish is a specialist and high risk activity, FSAS contract technical experts to assist the LFA in the approval of these premises. The contractor is responsible for setting the working protocols for the system's safe operation. The LFAs base their decision and scope of approval for the establishment upon this protocol and inspection report. Thereafter the contractor revisits the premises on an agreed risk-based frequency to ensure that parameters have not been altered. The LFAs will also visit the premises according to the risk-based system required under the principal Food Control Legislation, The Food Safety Act 1990 and associated Codes of Enforcement Practice.

The FSAS encourage and facilitate close working between the LFAs, contract technicians, the National Reference Laboratory for this area, The Centre for Environment, Fisheries and Aquaculture Science, and The Seafish Industry Authority whose depuration plant designs are endorsed for use within the United Kingdom.

The FSAS has produced LFAs guidance on the inspection of depuration facilities.

11.4.3 Control of production

EU 854/2004 (European Communities 2004a) allows harvesting of live shellfish to be prohibited where the LFA is satisfied that the consumption of the live shellfish is believed likely to cause a risk to public health. The LFA requires evidence with which it is satisfied to justify administering a Temporary Closure Notice. The Temporary Closure Notice has effect for a period of 28 days unless revoked earlier. In order to review the notice, ongoing evidence of a risk to public health is required.

The LFAs also have powers under The Food Safety Act 1990 to seize and detain any product unsuitable for consumption in so far as it may be injurious to health or not meeting food safety requirements as determined by a food examiner. The LFA also has responsibilities to conduct a sampling programme in addition to the Central Authority programme. In Scotland the local authority applies significant resource to supporting the Central Authority programme

and hence only a proportionate amount of supplementary LFA samples are taken during routine inspection visits to the site and depuration facility. These samples are mainly for verification purposes but will be used to support local enforcement action where appropriate.

11.4.4 Product traceability and product recall procedures

All products placed on the market are required to be labelled appropriately to ensure complete traceability. Every step in the chain requires full traceability. The gatherer of the shellfish is required to ensure that each consignment is accompanied to a dispatch centre, relay area or processing establishment with a valid registration document. A registration document is provided to the gatherer by the LFA and permits the movement of each consignment from the harvest area to one of the approved establishments as mentioned above. The LFA is charged with responsibility for ensuring that such requests for documents are dealt with and for ensuring that the shellfish is accompanied by the appropriate paperwork. The harvester has responsibility for ensuring that the shellfish is accompanied by the correct documentation to ensure full traceability. Any area subject to a Temporary Closure Notice is automatically prohibited from the issue of such documentation.

After treatment or packaging at a dispatch centre, live shellfish must be provided with a health mark to ensure that the original dispatch centre may be identified at all times during distribution and sale. The health mark must indicate the country of dispatch, species, date of wrapping and approval number of the dispatch centre.

The United Kingdom has a system of food alerts which are used to recall product placed on the market which is subsequently found to be non-compliant in some respect. Minor non-compliance issues are in the main covered by alerts of a lower priority rating, providing information other than those where there is a potential danger to public health. Food Alerts are brought to the attention of the Central Authority via the LFA who may discover an issue in the course of their local enforcement activity or by the harvester/processor on discovering an issue during quality control procedures, customer complaints or via the EU Rapid Alert for Food and Feed Stuffs system. The Food Alert is administered by the Central Competent Authority and distributed to the LFA for action. Where affected product may have been purchased by the consumer, Press Notices are arranged to bring the matter to the attention of any potential purchaser. All Food Alerts are additionally published on the FSA website. A formal Food Alert will only be used where necessary to protect public health and where a trade withdrawal on the part of the producer/processor is not appropriate or adequate.

11.5 COMMUNICATION OF OFFICIAL CONTROL RESULTS AND MITIGATION ACTIONS

Communication of official control results is very important. The LFA is responsible for managing shellfish harvesting production areas at local level and therefore sampling results from the programme are essential to assist them in determining whether any mitigation action is necessary. The LFA, along with the site harvester, is responsible for gathering the shellfish samples for the programme and for sending these to the laboratory contracted by the FSA for undertaking the flesh analysis. The FSA funds the analysis of these samples and manages the contract for this analysis. All results are communicated to the FSA weekly. These results are forwarded to the LFA as soon as quality checks are completed on the data. The LFA sends this data to individual site harvesters so that they aware of the results from their site.

Additionally, any samples which are found to be outside the current site classification limits are notified to the FSA immediately after the results are known. These are directed to the LFA immediately to allow them to conduct any necessary site investigation. In the majority of cases a further sample is taken to assist in determining whether there is an ongoing problem. Where a problem is identified other relevant investigatory agencies will be called to assist. In Scotland SEPA will be asked to investigate any potential sewerage contamination issues and to take appropriate action. Where an ongoing matter of public health significance is identified, the site will be subject to harvesting restriction by use of a Temporary Prohibition Order. This will remain in force until the LFA is satisfied that it is safe to resume harvest. In the event of such an incident the opinion of the National Reference Laboratory experts will be sought during the investigation. The National Reference Laboratory in these circumstances as funded by The Agency provides this service to LFAs. Temporary Closure Notices are lifted once perceived risk has been removed and two consecutive results taken at fortnightly intervals illustrate that results are within those acceptable for the area's classification status.

11.6 CONCLUSIONS – STRENGHTS AND WEAKNESSES OF THE FOOD STANDARDS AGENCY CURRENT SYSTEM

There are a number of strengths with the current system as operated by the FSAS. Firstly, all official control results are taken by dedicated Official Control sampling officers. The LFA employs these officers with grant-assisted funding

from the FSAS. These officers all receive benchmark training in all aspects of their duties, which is provided by the FSA with assistance from the National Reference Laboratory and the official control laboratories involved in the programme. Their function is to ensure that the areas are adequately controlled, that samples are gathered from correct locations and verified and that the programme is operated according to the requirements stipulated under EU 882/2004 (European Communities 2004b). This requires that official control samples are taken in the absence of bias and conflict of interest. The strength lies in having high quality officers who execute controls and ensure that samples are gathered according to timetable and from a verified location. The verification of samples is a fundamental part of the monitoring programme and allows all further regulatory decisions to be taken with confidence.

Training of all officers involved at local level is also a key strength to ensure that LFAs maintain the necessary expertise in this technical area. The FSA ensures a high skill base by provision of regular training for all officers including in the field of depuration plant inspection, sanitary survey process and management of outbreaks of infectious disease in relation to shellfish. A comprehensive package of Codes of Practice and Guidance documentation is provided to assist with uniform and consistent application of enforcement practices.

The handling of incidences related to shellfish is also subject to a formal system, which is documented and assists in the orderly management of such events. Formal communication channels are also strengths of the programme. These have been set up both locally and nationally to allow for adequate flow of information. These channels would, however benefit from the creation of written local action plans to support local action group activity for each area and this issue will be addressed in the near future.

11.7 REFERENCES

European Communities (2004a) Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. *Off. J. Eur. Communities* **L 226**, 25.6.04:83–127.
European Communities (2004b) Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. European Union*, **L 161**, 30/4/04:141.
European Communities (2006) Commission Regulation (EC) No. 1666/2006 of 6 November 2006 amending Regulation (EC) No 2076/2005 laying down transitional arrangements for the implementation of Regulations (EC) No 853/2004, (EC) No. 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council. *Off. J. Eur. Communities* **L 320**, 18.11.06:47–49.

12

Official control monitoring programmes for live bivalve molluscs – legislative and regulatory approaches: Canada

G. Sauvé

This chapter should be read in conjunction with chapters 10, 11, 13 and 14. Together these five chapters provide a detailed overview of existing legislation in a number of key countries whilst also highlighting the gaps, anomalies and principal issues that face those charged with the responsibility for monitoring and control programmes for live bivalve shellfish and their waters.

12.1 BACKGROUND AND CENTRAL COMPETENT AUTHORITY'S ROLE IN THE CANADIAN SHELLFISH SANITATION PROGRAMME

The Canadian Shellfish Sanitation Programme (CSSP) is Canada's federal food safety programme centred on providing assurance that bivalve molluscs (oysters, mussels, clams, and scallops) are safe for human consumption. The programme controls numerous areas along the coast of Canada and is jointly administered by three federal organizations: Department of Fisheries and Oceans (DFO), Canadian Food Inspection Agency (CFIA) and Environment Canada. The programme is based on control measures for water sanitation, biotoxin control, shellfish harvesting and processing.

The CSSP was originally developed as a result of an outbreak of typhoid fever traced to the consumption of contaminated oysters in the United States during the winter of 1924–1925. In response, Canada developed the first set of regulations (3 July 1925) under the Fish Inspection Act requiring that imported oysters be accompanied by a certificate to affirm that they were a "safe food product". In addition, the mutual concerns of Canada and the United States in protecting the public from the consumption of contaminated bivalve molluscs led to a formal shellfish agreement (30 April 1948) respecting sanitary practices for the shellfish industries of both countries. This bilateral agreement is still in effect today. The CSSP has continued to grow and evolve since that time.

The roles and responsibilities of the CSSP partners can be defined generally as follows:

Environment Canada is the lead agency with regard to water quality and classification of shellfish growing areas. Environment Canada's role is to assess environmental conditions that affect the sanitation of shellfish growing areas.

The DFO is the lead agency with regard to the controlled relaying, depuration and harvesting of shellfish from classified growing areas. DFO is responsible for the enforcement of closure regulations (posting and patrolling closures) and enacting the opening and closing of shellfish growing areas under the authority of both the Fisheries Act and Management of Contaminated Fisheries Regulations.

The CFIA is the lead agency with regard to the handling, processing, marketing, import and export of shellfish and liaison with foreign governments. CFIA is also responsible for the management of the marine biotoxin monitoring programme and any other microbiological monitoring programme not described under Environment Canada's responsibilities.

12.1.1 Overview of programme

Canada currently has over 1000 classified shellfish harvesting production areas that cover more than 21 000 km^2 (Table 12.1).

Table 12.1 Status, location and extent of Canadian shellfish harvesting production areas in 2003. Figures are in km^2

	Approved	Closed	Conditionally Approved	Total
Atlantic	4185	2152	80	6417
Quebec	2265	1895	270	4430
Pacific	8925	1046	195	10 166
Totals	15 375	5093	545	21 013

Source: Canadian Food Inspection Agency

The main species exploited are the scallops (*Placopecten* spp., *Chalmys* spp.), clams (*Panope* spp., *Mya* spp., *Saxidomus* spp., *Mercenaria* spp., *Mactromeris* spp., *Spisula* spp. *Siliqua* spp.), mussels (*Mytilus edulis*) and more recently, oysters (*Crassostrea gigas*, *C. virginica*, *Ostrea edulis*).

The approximate quantity and value of cultivated and wild harvested bivalve molluscs produced in Canada during 2005 are indicated in Table 12.2.

Table 12.2 Quantity (t) and value of Canadian-produced bivalve molluscs for 2005

Species	Quantity (t)	
	Wild fisheries	Aquaculture
Clams	28 497	1831
Oysters	3245	12 957
Scallops	57 176	61
Mussels	111	22 930
Other	–	832
Value (Canadian dollars)	146 million	67 million

Source: Department of Fisheries and Oceans, 2005

12.2 OFFICIAL CONTROL MONITORING PROGRAMMES MANAGED BY THE CSSP PARTNERING ORGANIZATIONS

The legal authority for the CSSP is provided by the Fisheries Act, Management of Contaminated Fisheries Regulations, the Fish Inspection Act and Fish Inspection Regulations. These Acts and Regulations enable the organizations to classify all actual and potential shellfish growing areas for their suitability for shellfish harvesting on the basis of sanitary quality and public health safety.

This authority also allows the responsible organizations (DFO, Environment Canada and CFIA) to:

- control the harvesting of shellfish from closed areas;
- regulate and supervise relaying, transplanting, cleansing and replanting;
- restrict harvesting of shellfish from actual and potentially affected areas in a public health emergency;
- prevent sale, shipment or possession of shellfish from unidentified sources;
- certify, inspect and determine the sanitary compliance of the operations of each shipper or processor;
- regulate the shipping conditions and labelling requirements for shellstock;
- regulate the export, import, processing, packaging, shipping storage and repackaging of shellfish;
- regulate the controlled purification of shellstock;
- suspend operations or decertify shellfish processors;
- evaluate laboratories performing shellfish analyses;
- collect samples and conduct appropriate bacteriological, chemical and physical tests to determine product quality; and
- prohibit the export or possession of shellfish from, for example, unidentified sources and uncertified dealers.

The Management of Contaminated Fisheries Regulations authorize the Regional Director General of the DFO to issue orders prohibiting harvesting of fish (finfish and shellfish) from areas where any kind of contamination or toxicity is present to an extent to be of public health significance.

Environment Canada administers the pollution abatement Sections 36–42 of the Fisheries Act which control the deposition of any deleterious substances to water frequented by fish or affects the use by man of fish that frequent that water.

12.2.1 Classification of new shellfish production areas

A major component of the CSSP is the identification of safe shellfish growing areas to permit commercial harvesting for the domestic market as well as for export. It is the responsibility of Environment Canada under the CSSP to:

- identify safe shellfish growing areas in Canada;
- promote pollution prevention, regulatory compliance, remediation and restoration of shellfish growing areas, together with federal/provincial/municipal agencies and other stakeholders;

- ensure proper application of prescribed analytical and reporting procedures including adequate quality assurance and quality control of the laboratory-generated data in Environment Canada laboratories, private laboratories (approved in accordance with the CSSP Manual of Operations) and laboratories under contract to Environment Canada; and
- ensure proper application of prescribed sampling procedures by qualified parties, including adequate quality assurance and quality control of the collected samples.

Shoreline surveys and bacteriological assessment of the overlying waters form the basis of the sanitary survey. The programme operating protocols require each harvesting area (including aquaculture sites) to undergo a comprehensive survey and risk assessment.

Areas are initially classified through comprehensive surveys to determine the sources of point and non-point pollution and the degree and extent of contamination. That may require using hydrographical and dye release studies, outfall modelling and sewage treatment evaluations. Classifications are based on the analysis of 15 sampling runs performed at random dates over a year and taking into account adverse pollution conditions. Each run entails withdrawing water samples at stations chosen to indicate the impact of any identified pollution source and to help set the final area boundaries.

Survey results and recommendations for classification are presented to CSSP partners for review and discussion. Environment Canada manages the information on databases and computer files that are GIS based. Final area classification and boundaries are decided upon by consensus and are implemented by DFO (also see section 12.2.3).

12.2.2 Classification of live bivalve mollusc production areas

12.2.2.1 Annual classification process overview

Re-surveys are conducted regularly to determine if sanitary conditions have undergone significant change. Change in pollution source conditions is evaluated in all approved growing areas annually by means of a formal reappraisal conducted both in the office and in the field. Environment Canada is in the process of implementing five sampling runs annually in all non-remote areas.

A complete re-evaluation of each approved area is conducted at least once every three years. This evaluation includes the field review of pollution sources, analysis of at least the last 15 water samples from each key station and other field works as deemed necessary to determine the appropriate classification for

the area. A minimum of five sampling runs are undertaken at every station during a re-evaluation. Results from annual reviews and triennial re-evaluations are documented in reports for each area.

12.2.3 Identification of production and relaying areas

In Canada, the need to delineate growing areas is based on requirements made in the Fisheries Act and fisheries regulations that specify, when closing an area for fishing, it must be precisely described. In the case of shellfish growing areas, the boundaries are proposed by Environment Canada based on the distribution of contamination as revealed by water testing results. The proposed boundaries are then reviewed by the other partner organizations to make sure it will not create problems, be it a popular clam bed being split over two or more official areas or difficulty identifying the boundaries in the field when performing surveillance of a closed area or for other considerations.

Shellfish growing areas used for aquaculture are managed by a leasing system that is under the authority of regional (provincial) authorities. When filling in a request form, the proponent is required to provide precise geographic coordinates (latitude and longitude) that are reviewed, when located in the marine environment, by all relevant ministries and departments to make sure it does not contravene any regulations governing land (sea bottom) or water usage. After final approval the new growing area will be classified by Environment Canada.

12.3 SUMMARY OF CANADIAN MONITORING PROGRAMMES

The surveyed waters are located in three major regions of Canada (Atlantic, Quebec and Pacific) within which management is done according to provincial boundaries. There are more than 15 000 stations used in the surveys to classify and re-evaluate over 21 000 km^2 (Table 12.3). However, a major part of Canadian waters are not surveyed for one or more of the following reasons: lack of resources, distances, climatic conditions or limited resource use.

The CSSP categories of classification are:

Approved

Areas where harvesting for direct consumption of shellfish is permitted. The area is not contaminated with faecal material, poisonous or deleterious substances or marine biotoxins to the extent that consumption of the shellfish might be hazardous; and the median or geometric mean (GM) faecal coliform most

probable number (MPN) of the water does not exceed 14/100 ml, and not more than 10% of the samples exceed a faecal coliform MPN of 43/100 ml for a five-tube decimal dilution test. Approved classification is required when a growing area is used for relaying purposes or used as a source of clean untreated water in an approved depuration system.

Table 12.3 Number of samples taken annually and number of sampling stations in the respective Canadian shellfish production regions

	Atlantic	**Quebec**	**Pacific**
Number of stations	6500 marine sites ± 50 freshwater/y	3500 marine 500 freshwater	3500 marine 1900 freshwater
Number of samples[*]	12 000 marine ± 50 freshwater 30 wastewater	4000 marine water 500 fresh water	5000 marine and freshwater

[*]Water samples are analysed in accredited laboratories for faecal coliforms using a validated most probable number (MPN) procedure. Water analysis normally begins within 8 h of sampling with no samples accepted after 24 h or at a temperature over 10°C.
Source: Canadian Food Inspection Agency

Conditionally Approved

Areas where, during those time that harvesting is permitted, meet all of the requirements of an "approved" area. Conditions which preclude harvesting in areas designated "Conditionally Approved" must be easily identified by routine measurement and reporting, and be predictable and/or controllable. A management plan must be developed and signed by all parties involved.

Closed

Direct marketing of shellfish is not allowed.

Restricted for controlled purification (depuration)

The median or GM faecal coliform MPN of the water does not exceed 88/100 ml, and not more than 10% of the samples exceed a faecal coliform MPN of 260/100 ml for a five-tube decimal dilution test.

Restricted for relaying

Areas within closed areas in which the median faecal coliform MPN of the water exceeds 14/100 ml, and/or more than 10% of the samples exceed a faecal coliform MPN of 43/100 ml for a five-tube decimal dilution test. These areas must not be within a prohibited area.

Prohibited area

Prohibited areas are distinct areas or areas within closure that are prohibited to shellfish harvesting for any purposes. Such areas include 300 m minimum exclusion zones around major point source discharges and 125 m minimum exclusion zones around wharves.

In order to support and complement the classification work performed by Environment Canada, the CFIA implements additional shellfish monitoring:

(1) as part of CFIA's national sampling plan, end-product shellfish samples are taken during audits at processing facilities to verify that the bacteriological standard/action levels (Canadian Food Inspection Agency, 2008a) are being met (see section 12.3.3);

(2) when samples from processing facilities exceed bacteriological guidelines, shellfish samples may be taken from the harvest area as part of follow-up investigation;

(3) as part of the verification of a relaying or a depuration process;

(4) before the reopening of areas classified "conditionally approved"; and

(5) in emergency situations which are not predictable or controllable under routine monitoring (such as natural or operational events such as hurricanes, flooding, emergency oil, toxic chemical and major sewage spills) (refer to CSSP Manual of Operations, Chapter 2; Canadian Food Inspection Agency, 2008a).

In addition to area classification based on marine water analysis, another major requirement of the CSSP is the marine biotoxins monitoring programme: the establishment of this programme is the responsibility of the CFIA.

12.4 RESPONSIBILITIES AND INTERACTION WITH OTHER AGENCIES

12.4.1 Interaction with the Local Food Authority

In Canada, fisheries management is the responsibility of the federal government which has delegated part of it through specific agreements to provincial authorities. One exception is aquaculture which falls under the purview of both federal and provincial legislations. Hence wild shellfish harvesting is solely a federal responsibility while shellfish aquaculture is shared between both federal and individual provincial authorities. Because of the specific factors found in each region of the country, federal CSSP specialists from the three partnering

organizations are located in each province where the program is implemented as well as at headquarters. This type of structure requires good coordination.

To that effect, CSSP co-ordination is achieved through regularly scheduled meetings of National and Regional Interdepartmental Shellfish Committees (NISC and RISC). The NISC reviews reports on programme operation, exchanges technical information, discusses national policy issues, considers proposed regulations and amendments and deals with issues on which there is no resolution at the regional level. The RISC's are composed of representatives from the three federal partners, as well as from appropriate provincial government ministries. Other stakeholders are invited, when needed, to present their position on specific topics. These committees make recommendations on the classification of shellfish growing areas.

Since Canada is a major exporter of molluscan shellfish to the United States and, to a lesser extent, to the European Union, CSSP has been subject to audit by the United States Food and Drug Administration (US FDA) and by the Food and Veterinary Officers of the European Commission to ensure that it is equivalent to the United States programme or the relevant European Directives.

12.4.2 Interaction with consumers

The DFO has as its principal goal proper fisheries management and has, within the CSSP, the legal authority under the Management of Contaminated Fisheries Regulations to close shellfish growing areas based on information and recommendations from CFIA and/or Environment Canada. In its role of enforcement of closures, DFO posts notices and makes public announcements to inform harvesters who may be commercial fishers or recreational gatherers. The DFO also offers toll-free phone numbers to reach answering machines with updated information on opened harvest areas. It also patrols growing areas to ensure that there is no illegal harvesting of unsafe product. With regard to this role, the DFO answers consumer's questions with the help of Environment Canada or CFIA when explanations are requested on the particulars of the monitoring programmes.

As the federal regulator of food, the CFIAs foremost responsibility is to enhance the safety of Canada's food and protect the health of Canadian consumers. To ensure the safety of bivalve molluscs as food, the CFIA is responsible for regulating (under the Fish Inspection Regulations) shellfish import and export, processing, packaging, labelling, shipping, certification, storage and product quality. Fulfilment of those responsibilities implies limited interaction with consumers but frequent communication with industry representatives.

The most frequent interactions with consumers are by the provincial and local food inspection authorities that inspect retail stores and regulate all local shellfish dealers and processors that sell only within their province.

12.4.3 Interaction with industry

The molluscan shellfish industry is mainly comprised of commercial harvesters, processors and their respective associations, as well as of brokers and importers. It is the policy of the CSSP partners to meet with any party that has made a request with regard to the administration, procedures or decisions made under the CSSP.

As the resource management and licensing authority, the DFO issues commercial fishing licenses to individuals who want to harvest molluscs from open growing areas for direct marketing or to supply processing establishments. This activity is linked to annual consultations with stakeholders where resource abundance and fishing pressure are discussed in order to adapt the fishing seasons or quotas to availability of the resource.

Similarly, the DFO has the authority to grant special fishing licenses to promoters (usually processors or aquaculturists) who want to harvest shellfish in "Conditionally Approved" or "Restricted for Purification" or "Restricted for Relaying" areas as long as they accept to enter into a formal agreement that specifies all the measures that will be taken to ensure the molluscs marketed are safe. These agreements are also signed by other CSSP partners and many other parties involved such as the waste water treatment plant operator, the municipality, the provincial authority and the testing laboratory.

In protecting the sanitation of bivalve molluscs destined for food markets, the CFIA inspects establishments engaged in the processing, holding and export of shellfish and issues certificates of registration to those plants which meet federal regulatory requirements. In concert with the issuing of registration certificates, CFIA inspection staff also conduct audits to assess the implementation and effectiveness of the Quality Management Plans (QMPs) (HACCP- based quality system) implemented by each registered establishment. In 2004, there were approximately 230 registered shellfish processing establishments operating in Canada.

A registered producer who wants to export will need an export certificate (except for shellfish destined to the USA if the Canadian producer is on the US FDA's ICSSL), issued by a CFIA inspector, that certifies the exported lots of product comply with the foreign country's requirements. For shellfish imported into Canada, CFIA is responsible for the licensing of importers and inspecting imported shellfish. Canada accepts live or raw bivalve molluscs only from

countries that have an agreement with Canada (Canadian Food Inspection Agency 2008b).

12.5 MITIGATION MEASURES

12.5.1 CSSP measures when molluscs exceed microbiological guidelines

Domestic molluscan shellfish (except scallop adductor muscles) or their derived products, whether fresh or frozen, are considered satisfactory when they are harvested from an "Approved" or "Conditionally Approved" area and *E. coli* (for end-of-line product) or faecal coliforms (product prior to processing) counts conform to the current CFIA Bacteriological Guidelines for Fish and Fish Products (Canadian Food Inspection Agency, 2008a).

When a shellfish sample taken at the plant fail the Bacteriological Guidelines, a QMP review will be performed to verify that the processor ensures that all shellfish received were harvested from open areas and that all plant records, monitoring and corrective actions have been properly implemented and recorded.

If, after a product failure, a review of the plant's QMP indicates that the plant is in control of its operation, 10 sample units will be taken by CFIA at the implicated harvest area for faecal coliforms analysis. If there is reason to believe that the harvest area classification is not current, the area may be closed without on-site sampling.

If the results of the sample units' analysis were microbiologically acceptable, the suspect area would be targeted for microbiological sampling during the next QMP verification. Repetitive failures will result in QMP being rated "ineffective" which may result in the establishment registration being suspended or revoked.

If greater than 10% of the samples units analysed exceed 230 faecal coliforms per 100 g (or one sample exceeds 2300), the area shall be closed. Re-sampling may be performed by the CFIA after a minimum of seven days and if results are acceptable the area shall be re-opened. The area may be kept closed and Environment Canada requested to re-evaluate the area (classification) as survey schedules permit.

12.5.2 Treatment measures

When an area is too contaminated to allow direct marketing, be it classified as "Restricted for Controlled Purification" (depuration or relay) or "Conditionally Approved" but in its temporarily closed status (condition for allowing direct

marketing is not met), DFO may issue, under the Management of Contaminated Fisheries Regulations (Department of Justice Canada 1990), a special fishing license to allow harvesting if an acceptable purification process is submitted that will render the molluscs safe. The CFIA, which is responsible for evaluating the proposed purification processes, recognises different acceptable procedures that will achieve this.

12.5.2.1 Depuration

The CFIA requires all depuration establishments to be registered and to develop a QMP (HACCP) that will be regularly audited. Above the QMP requirements, the CFIA also requires that depurators meet specific criteria in relation to the facility standards, processing water standards, cleaning systems, tanks, processing containers, water treatment, storage, and laboratory analysis and other operational controls and procedures which ensure that acceptable product reaches the market (Canadian Food Inspection Agency 2008a). More specifically, the treated product must meet more stringent microbiological standards: as an example, each lot of depurated oysters must have a geometric mean (five sample units) of no more than 50 faecal coliforms per 100 g with no more that 1/5 units over 100 cfu/100 g and none over 170 cfu/100 g.

12.5.2.2 Relaying

The CSSP Manual of Operations (Canadian Food Inspection Agency, 2008a) details all control measures that must be implemented to assure the safety of molluscs relayed either for short (less than 14 days) or long term (more than 14 days). Final product microbiological standards are the same as for depuration.

12.5.2.3 Other treatments

In some circumstances, it may be acceptable to heat process (for example canning) molluscs that were harvested in areas that do not meet the "Approved" area criteria.

12.6 PRODUCT TRACEABILITY AND PRODUCT RECALL PROCEDURES

In case of an outbreak of disease attributable to shellfish, it is necessary that health departments and other appropriate local and federal agencies be able to determine the source of contamination and thereby to prevent any further outbreaks from this source.

In order to achieve this, the CSSP requires that molluscan shellfish are labelled with specific information showing that they were harvested by licensed diggers and shipped and processed by registered dealers. This information makes it possible to trace product back through the distribution system to the processor and to the growing area in the event that the shellfish are associated with a disease outbreak.

As per the Fish Inspection Regulations (FIR), an establishment that processes molluscan shellfish for export will be, upon request, registered if it meets, among other things, the following two requirements:

The applicant must develop and submit a quality management programme (QMP) [HACCP based] as described in other FIR sections.

The applicant must meet the applicable requirements set out in the Canadian Shellfish Sanitation Programme Manual of Operations (Canadian Food Inspection Agency, 2008a).

An operator of a registered establishment shall maintain, at an address in Canada and for not less than three years, a record of:

(1) the common name of the shellfish,
(2) the quantity by weight of the shellfish delivered to the establishment,
(3) the location where the shellfish was harvested,
(4) the date on which the shellfish was harvested,
(5) the name, address and telephone number of the person who harvested the shellfish,
(6) the method of transport and the date on which the shellfish was delivered to the establishment, including details of the method and conditions of storage before and after delivery,
(7) the manner in which, and the date on which, the shellfish was processed in the establishment, and
(8) the date on which the shellfish was shipped from the establishment and the name and address of the person to whom it was shipped.

In addition to any other labelling requirements, every container or the label thereon shall be correctly and legibly marked to indicate, in the case of bivalve molluscs in the shell, the date of processing and the location from which the bivalve molluscs were harvested.

More product-specific measures are described in the CSSP Manual of Operations (Canadian Food Inspection Agency, 2008a). For example, depurated shellfish require an increased level of control compared to shellfish from approved areas because of the increased potential for contamination. These controls must include packaging and labelling that will serve to help identify the depuration

cycle of each harvest lot and to deter illegal co-mingling of undepurated shellfish with depurated shellfish. Such controls include prohibition against co-mingling of harvest lots during packing, tags that identify the shellfish as being depurated, and a prohibition against repackaging after the shellfish leave the depuration plant.

Local (provincial) authorities have similar regulations that will specifically require dealers, retailers as well as restaurants and institutions, to maintain precise identification of molluscs for some time, even after sale or use.

Should a marketed shellfish product be linked to a disease outbreak, the CFIA, under the Food and Drugs Law and Regulations has the power to order a recall that may, according to the risk level identified, be accompanied by a public notification. An imported product shown to be in violation of Canadian regulations will be placed on the Alert List until four consecutive shipments are shown to be in compliance.

12.7 EMERGENCY CLOSURE

An ''Approved'' shellfish harvesting area may be closed when it is suspected that shellfish may be contaminated as a result of an emergency situation which is not predictable or controllable under a routine monitoring programme. These emergency situations may include natural or operational events such as hurricanes, flooding, and emergency oil, toxic chemical and major sewage spills.

When a representative of the shellfish control agencies (DFO, Environment Canada, CFIA and appropriate provincial department(s)) is advised of an emergency situation he/she will recommend to DFO a precautionary closure that will cover all growing areas that may be affected.

Environment Canada and/or CFIA will advise the DFO if there is a need to rescind or modify the scope of the precautionary shellfish closure upon receiving more detailed information from the reporting agency. The DFO will modify the closure accordingly.

The closure will remain in place for at least seven days. At this time, Environment Canada and/or CFIA will evaluate the situation and advise DFO on the status of the closure, as well as a plan for continued evaluation. Once the bacteriological and chemical quality of the water and shellstock is satisfactory, CFIA and Environment Canada will advise DFO to reopen the area and notify the provinces of their findings and any further follow-up.

12.8 CONCLUSIONS – CSSP CHALLENGES

The management by three separate governmental organizations of a complex programme that covers such a large country with diverse populations poses

many challenges. For example, coordination of efforts needs to be enhanced to ensure that regular and ongoing interactions are held among each of the three CSSP partners to jointly assess the success, efficiencies and output in delivering the CSSP in the interest of food safety.

There are increasing demands on the programme from a growing shellfish aquaculture industry, new technologies, recreational use and new and developing shellfisheries. This creates the need for increased monitoring and CSSP expansion into new areas. The emergence of new shellfish fisheries managed by native populations in remote and distant regions of Canada is another challenge for the programme.

There is concern over increasing urbanization, sanitary and biotoxin closures and accountability for toxic substances. Closures of harvesting areas will increase with the continued coastal development, unless adequate wastewater treatment measures are adopted and non-point sources are reduced.

There are challenges to stay abreast of the most up to date scientific findings and technological advancements and, furthermore, incorporating them into the CSSP programme. Similarly, more research is needed to establish baseline knowledge on topics such as shellfish resources, effectiveness of conservation efforts, pathogens associated with shellfish, decontamination, updating of classifications and standards. Lastly, public communication and education relating to shellfish safety needs to be enhanced.

12.9 REFERENCES

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13

Official control monitoring programmes for live bivalve molluscs – legislative and regulatory approaches: New Zealand

P. Busby

This chapter should be read in conjunction with chapters 10, 11, 12 and 14. Together these five chapters provide a detailed overview of existing legislation in a number of key countries whilst also highlighting the gaps, anomalies and principal issues that face those charged with the responsibility for monitoring and control programmes for live bivalve shellfish and their waters.

13.1 BACKGROUND AND CENTRAL COMPETENT AUTHORITY'S ROLE IN SHELLFISH CONTROLS: THE NEW ZEALAND FOOD SAFETY AUTHORITY

The New Zealand Food Safety Authority (NZFSA) was established as a Public Service department on July 1 2007. Previously, it was a semi-autonomous body attached to the Ministry of Agriculture and Forestry, made up of food safety officials from the Ministry of Health and the Ministry of Agriculture and Forestry Food Assurance Authority. The NZFSA reports directly to the Minister for Food Safety. NZFSA's priority is safe, nutritious and suitable food. It protects the health of New Zealanders and recognizes that the export of food and food-related products forms the basis of the country's economic well-being.

The NZFSA has two key roles:

- to protect and promote public health and safety, and
- to facilitate market access for New Zealand's food and food-related products.

To fulfil these roles, the NZFSA administers legislation and government policies that cover production of food and related products and the sale of these in New Zealand. The NZFSA also performs the Government "Competent Authority" regulatory duties of controls and assurances covering imported and exported foods.

The NZFSA is charged with ensuring a safe and suitable food supply by:

- providing the Minister for Food Safety with policy advice on food and food-related issues;
- setting standards related to food safety and suitability as required by legislation, other policy or market access;
- implementing programmes that ensure all safety and suitability requirements are met;
- enforcing legislative requirements;
- providing official assurances to importing countries and attesting that their safety and suitability requirements are met;
- assuring continued economic health through trade and commerce in food-related products; and
- ensuring effective communication with stakeholders.

13.1.1 The New Zealand bivalve molluscan shellfish industry

Growing and harvesting bivalve molluscan shellfish (BMS) for trade is an important part of the New Zealand economy and is seen as a vital part of New Zealand's future by the Government. Some 80% of BMS grown in New Zealand is exported to over 60 countries, therefore, it is important that the New Zealand have in place regulatory BMS standards that encompass international best practice.

New Zealand currently has 87 classified BMS growing areas with most being in the aquaculture group. Approximate quantities (tonnes) commercially harvested annually in New Zealand are shown in Table 13.1.

Table 13.1 Approximate annual quantities (tonnes) of commercially harvested bivalve molluscs in New Zealand (2006 figures – total value US$200M)

Species	Quantities (t)
Greenshell™ Mussels (*Perna canaliculus*)	97 000
Pacific Oysters (*Crassostrea gigas*)	2800
Dredge Oyster (*Tiostrea chilensis*)	2000
NZ Littleneck Clams (*Austrovenus stutchburyi*)	3200
Surf Clams (variety of species)	600
Scallops (*Pecten novaezelandiae*)	1000
Southern Queen Scallop (*Chlamys delicatula*)	800

Greenshell™ Mussels and Pacific Oysters are aquaculture BMS, generally cultured in between five- and seven-hectare farms. However, the difficulty in obtaining more water space has resulted in applications/consents for between two and ten thousand hectare farms in offshore (between five and ten kilometres) areas. Some farms may contain multiple species of BMS.

13.1.2 New Zealand shellfish programme culture

In setting the new BMS standards, two key factors had a significant influence. Firstly, in 1980 a Shellfish Memorandum of Understanding was signed between the New Zealand Ministry of Agriculture and Fisheries and the United States Food and Drug Administration (US FDA) and this delegated the responsibilities for classification and monitoring of BMS areas to the Public Health Division of the Department of Health. This work, along with the marine biotoxin control is currently performed by Health Protection Officers (warranted as Animal Product Officers under the Animal Product Act, 1999) employed by Public Health Units of District Health Boards (Crown Entities).

Secondly, in 1987, the New Zealand Government implemented a mandatory cost recovery regime, that required that the shellfish industry pay for all costs involved in meeting regulatory requirements including the sanitary survey, classification, monitoring and laboratory analyses requirements for the BMS programme.

13.2 OFFICIAL CONTROL MONITORING PROGRAMMES FOR BMS

In 1991, New Zealand developed its first shellfish safety standards under the Meat Act 1981. When the Animal Products Act 1999 superseded the Meat Act 1981, the BMS standards underwent a complete revision and new requirements came into effect on 1 June 2006 as the Bivalve Molluscan Shellfish Regulated Control Scheme (BMSRCS), comprising the Animal Products (regulated Control Scheme-Bivalve Molluscan Shellfish) Regulations 2006 and the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006.

13.2.1 BMSRCS regulations

The BMSRCS Regulations state that the prime purpose of the BMSRCS is:

"to identify, monitor, evaluate and manage the risks associated with:

the commercial growing, harvesting, sorting and transporting of BMS intended for human consumption; and

other related activities or conditions affecting the suitability for processing or fitness for intended purpose of BMS".

The Regulations also state that the BMSRCS applies to:

"all activities involved in the growing harvesting, sorting and transporting BMS for commercial purposes up until the time when:

the BMS are received by a wholesaler or retailer or sold direct to the consumer, in the case of BMS that do not undergo primary processing; or

the BMS undergo primary processing, in any other case; and

the collection and analysis of samples of BMS and associated things for monitoring under this scheme".

Part 2 of the Regulations describes provisions for the suitability of BMS, vessels and vehicles used for transporting BMS; standards for persons handling

BMS; identification, labelling and record keeping; obligations of growers and obligations of harvest, relay, transport, sorting shed and depot operators. The requirements for classification, sanitary survey, monitoring and procedures for opening and closing areas are also described. Part 3 addresses the registration, permits and listings of BMS-related operators, while Part 4 addresses miscellaneous provisions including offences.

13.2.2 BMSRCS specifications

The specifications are classed as tertiary law, quite prescriptive and are divided into 19 parts comprising: preliminary provisions, their interpretation, BMS growing areas, growing area classification and status, relaying of BMS, wet storage (at sea) of BMS, marine biotoxin control, sampling, control of BMS harvesting, requirements for harvest operators, vessels and vehicles, health of personnel, sorting sheds and depots, transport of BMS, microbiological risk management, marinas, BMS laboratories, calibration and record keeping.

13.2.3 Sanitary survey and classification

A sanitary survey must be completed prior to the classification of a BMS growing area. The sanitary survey includes:

- a shoreline survey;
- a survey of the bacteriological quality of the water in the growing area and adjacent areas;
- a survey of the bacteriological quality of BMS in the growing area;
- an evaluation of the effect of any hydrographic, meteorological and geographic characteristics of the growing area and catchment;
- an analysis of the data from the shoreline survey, bacteriological survey, and the hydrodynamic, meteorological and geographic evaluation;
- a determination of the appropriate growing area classification and, for Conditionally Approved and Conditionally Restricted growing areas; and
- a determination of the harvest criteria.

The BMSRCS requires that the Animal Product Officer:

- determine the boundaries, based on catchment area topography, of each shoreline survey area;
- conduct an in-the-field investigation which identifies properties with the potential to have an impact on the growing area;

- identify, investigate and evaluate all potential sources of pollution which may affect a growing area;
- determine the distance from each potential pollution source to the growing area;
- determine the impact of each pollution source on the growing area under normal and adverse pollution conditions;
- document for each potential pollution source in the catchment identified likely to affect a growing area:
  a. the location and GPS co-ordinates or other acceptable identification of the pollution source on a comprehensive map of the growing area catchment; and
  b. the determination that the pollution source has a direct or indirect impact on the growing area;
- evaluate all farms with animals, including the number and types of animals, the access of animals to watercourses and the type and effectiveness of animal waste treatment systems;
- determine the effects of domestic and wild animal populations, including resident or migrating populations, deer, seals and penguins, including estimation of numbers and seasonality;
- evaluate all lakes, drains, ditches, streams, rivers and other watercourses in the catchment for potential effects on the growing area;
- assess the reliability and effectiveness of sewage or other waste treatment systems that may affect the growing area; evaluate each human waste management system, including septic tanks, and determine if their intended purpose is met;
- evaluate the potential for cyanotoxin contamination of BMS in the growing area; and determine if toxic substances are likely to adversely affect the growing area; and include the findings of the shoreline survey in the sanitary survey report.

Based on data from the above each growing area is classified as Remote Approved; Approved; Conditionally Approved; Restricted; or Conditionally Restricted. The bacteriological standards for these classifications are described in section 13.2.6. Any upward revision of a growing area classification must be supported by a sanitary survey conducted in the twelve months prior to the reclassification.

When a growing area does not comply with the bacteriological standards for its classification, it must be closed immediately and the classification reviewed. A growing area must also be closed immediately following a public health

emergency such as a broken sewer pipe, the detection of pathogens, a toxic substance spillage, storm, flood, or any other event which may affect the public health quality of the growing area water or BMS.

13.2.4 Annual review of the sanitary survey

Although quite prescriptive, this risk assessment approach is critical to provide assurance that all potential pollution sources in the BMS catchment are identified and evaluated to provide for suitable risk management through the growing area classification process. The BMSRCS requires that, on an annual basis, and within 60 days of the anniversary date of the sanitary survey report, an Animal Product Officer must review the sanitary survey and classification of each growing area and the review must include:

- a field observation and evaluation of the pollution sources identified in the sanitary survey and their performance standards, if any. This may include a drive-through survey, observations made during sampling and information from other sources;
- the identification of any new pollution sources and an evaluation of their effect on the growing area;
- an evaluation of the quality of the growing area water and BMS in the growing area with respect to the bacteriological standards for classification;
- a review of the sampling activity;
- a summary of any heavy metal or toxic substance analyses performed;
- a review of the adverse pollution conditions identified in the sanitary survey;
- for Conditional areas, an evaluation of compliance with the management plan, a determination of the adequacy of reporting of failure to meet performance standards (such as for sewage treatment plants) and a review of the cooperation of the agencies and persons involved;
- the taking of any necessary action by the Animal Product Officer, such as adjustment of harvest criteria, reclassification, additional water or BMS sampling, hydrographic or any other work considered necessary by the Animal Product Officer to maintain the sanitary survey; and
- the written findings, evaluations and recommendations, including a determination that the existing classification and harvest criteria are correct or require changing.

13.2.5 Samples and sample stations

The location and number of sample stations must be adequate to allow the effective evaluation and routine monitoring of all actual and potential pollution sources that may have an impact on the bacteriological quality of the growing area. This must take into account the spatial and depth variability that may occur for each commercial BMS species. Provision is made for one species to be used as a bacteriological indicator for other species that may be commercially harvested from the growing area. The location of sample stations for new growing areas that are adjacent to, or extensions of, existing growing areas is specifically provided for.

The collection of BMS and water samples during a sanitary survey must provide adequate data to form a profile for periods defining adverse pollution conditions. The profile must address adverse meteorological, hydrographic, tidal, turbidity and seasonal conditions and point sources of pollution to ensure that the requirements for classification are met.

For new growing areas, a minimum of 30 water and 30 BMS samples collected under various environmental conditions over a minimum of 12 months must be taken to classify an area.

For classified areas, the bacteriological data for water and BMS taken over the last three years under adverse pollution conditions must be reviewed to confirm the classification, as part of the annual review.

The specifications provide for an option, which may be used only under specified conditions, to use a systematic random sampling strategy, rather than an adverse pollution condition sampling strategy. The conditions for using the alternate strategy are prescribed in a schedule to the specifications. However, since this option became available, only one growing area has chosen to use it.

13.2.6 Bacteriological standards for BMS and water

For Remote Approved, Approved and Conditionally Approved areas, the growing area water and BMS must meet the following bacteriological standards at each sample station in the growing area when it is open for harvesting:

- the faecal coliform median most probable number (MPN) of the water samples must not exceed 14 per 100 ml and not more than 10% of the samples must exceed and MPN of 43 per 100 ml; and
- the *E. coli* median MPN of the BMS samples must not exceed 230 per 100 g and not more than 10% of the samples must exceed an MPN of 700 per 100 g.

For Remote Approved areas, a minimum of two samples must be collected annually, for Approved areas a minimum of five samples must be collected annually and for Conditionally Approved areas, monthly samples must be collected. All such samples must be collected under adverse pollution conditions, by a certified sampler.

For Restricted and Conditionally Restricted areas the growing area water and BMS must meet the following bacteriological standards at each sample station when the area is open for harvesting:

- the faecal coliform median MPN of the water samples must not exceed 88 per 100 ml and not more than 10% of the samples must exceed 260 per 100 ml; and
- the *E. coli* median MPN for BMS must not exceed 4600 per 100 g and not more than 10% must exceed 14 100 *E. coli* per 100 g.

Five samples per year must be collected to maintain the Restricted classification and monthly samples must be collected to maintain the Conditionally Restricted classification. All samples must be taken under adverse conditions.

The specifications provide for the failure to take the monthly sample when environmental conditions such as rough seas occur and require that an additional sample be taken the following month. Special provisions are also made for unusual bacteriological events including exceeding the bacteriological standard for the area.

The 10% mentioned above is not considered adequate to protect public health when known meteorological or hydrological events that occur intermittently are shown to adversely affect growing water quality. The "percentage factor" is not intended to allow for variation in the data caused by changes in environmental conditions at the time of sampling. Rather, it is intended for use with a data set collected under uniform conditions and is intended to reflect the inherent variation of the MPN methodology, although the current "10% not greater than" levels allow a somewhat greater degree of variation that attributable to the MPN test alone.

The dilemma facing regulators is how to distinguish between the inherent variation of the MPN test and that resulting from intermittent environmental conditions that degrade water and BMS quality. It is not intended however, that BMS growing waters be classified that are polluted 10% of the time. In determining compliance with bacteriological standards, elevated bacteriological levels must not be associated with environmental conditions, particularly those that are described in conditional management plans as adverse pollution conditions.

13.2.7 Conditional classification

Growing areas may only be classified as Conditional when the sanitary survey demonstrated that:

- the growing area will be in the open status of the classification for a reasonable period of time, the factors determining this period are known, are identified in the sanitary survey or annual review, are predictable and are not so complex as to preclude a reasonable management approach;
- each potential source of pollution that may adversely affect the growing area is identified, evaluated and its effect on the growing area discussed in the sanitary survey; and
- the bacteriological quality of the growing area water and BMS correlates with environmental conditions or other factors affecting the distribution of pollutants into the growing area.

For each Conditional growing area, a written management plan must be developed by an Animal Product Officer prior to the classification and include studies that show the time interval necessary for the reduction of faecal coliform levels in the growing water and *E. coli* in the BMS back to background levels. A clear description of the procedures and methods for opening and closing areas must also be provided in the management plan. Among other requirements for the management plan for Conditional areas, it must be understood and agreed upon in writing by all the parties involved, such as the regulatory agency, the shellfish industry and persons involved in the management of sewage treatment plants. The failure of any one party to agree on the conditions in the management plan is sufficient reason to close the growing area.

13.2.8 Sewage management

When a sewage treatment plant outfall is situated in, or adjacent to, a growing area the surrounding area must be classified as prohibited. The determination of the size of the area to be so classified must include an assessment of the following:

- the volume, flow rate, location of discharge, performance of the wastewater treatment plant and the bacteriological quality of the effluent;
- the decay rate of the contaminants of public health significance in the wastewater discharged; and
- the characteristics of the receiving water (including bathymetry, current velocity, net transport velocity, water depth and volume, direction of

flow, water stratification, tidal characteristics, dilution rate and likely dispersion);

- the wastewater's dilution and dispersion and the time of waste transport to the area where the BMS may be harvested; and
- the location of the shellfish resources, classification of adjacent waters and identifiable landmarks and boundaries.

The specifications require that the size of the prohibited area be determined using computerized steady-state simulation models such as the US Environmental Protection Agency PLUMES Dilution Model for Effluent Discharges (USEPA 2003).

In relation to sewage events such as spills or pipeline breaks, the specifications require that the growing area be closed for a minimum of 28 days from the end of the event or for a greater or lesser time as determined by the regional shellfish specialist.

All BMS harvest vessels are required to have an acceptable marine sanitation device to contain human sewage and any sewage discharged from a vessel must not occur within 500 metres of the growing area boundary.

13.2.9 Pathogen management

Part 13 of the BMSRCS specification contains detailed requirements concerning the investigation and activities surrounding a BMS-related illness outbreak involving two or more persons. Epidemiological association between the outbreak and BMS consumption is defined and used to initiate the investigation and subsequent actions. Specific requirements are also described for action to be taken when pathogens are detected in BMS without an associated illness.

13.3 CONCLUSIONS

In drafting the new BMS safety regulations and specifications, significant effort was put into determining international best practice in the various fields. As a result, *E. coli* testing of BMS was added to the classification requirements to provide a better assurance of safety and other parts of New Zealand's historic standards were also updated. However, irrespective of what the legislative requirements are for a particular country, they are of little use unless they are effectively implemented. The use of Health Protection Officers, with their public heath qualification and experience, to perform the sanitary survey and classification of BMS growing areas has ensured that the risk assessment and

risk management approach of the BMSRCS provides the necessary food safety assurance for New Zealand shellfish.

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14

Current management practices

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Chapters 11–13 of this monograph provided descriptions of the regulatory systems, responsible authorities and decision-making processes managing the bivalve shellfish industries in Scotland, Canada and New Zealand. These contributions gave us a valuable insight into how developed economies with significant shellfish industries approach their practical management responsibilities. In Chapter 10, Murray and Lee described how there are effectively two accepted approaches to bivalve shellfish regulation in use across the world – the United States approach via its National Shellfish Sanitation Programme (NSSP) and the European Union (EU) approach. Murray and Lee further pointed out that there are many countries that have no management schemes in place.

In the present chapter we will explore further the differences and similarities between the legislation in practice in Scotland, Canada and New Zealand, and then go on to look at the situation in a major multi-national coastal region which has nation states occupying a range of developmental scenarios (i.e. the Mediterranean) and in two of the world's most rapidly developing economies

(i.e. China and India). This should provide the balance required in assessing what is desirable and what is achievable in management practice of bivalve shellfish industries, providing the reader with a more holistic view than merely restricting observations to systems in developed countries that have access to adequate resources.

14.1 APPROACHES TO SHELLFISH MANAGEMENT IN SCOTLAND, CANADA AND NEW ZEALAND – REPRISE

Management and regulatory procedures in the shellfish industries in Scotland, Canada and New Zealand were comprehensively dealt with in chapters 11, 12 and 13 respectively. These countries adhered closely to the previously described models (chapter 10) inasmuch as Scotland, as an EU member, faithfully applies the EU approach; Canada, with its close relationship to the United States, applies a variant of the United States NSSP approach and, most interestingly, New Zealand with its extensive shellfish export industry applies what is, in effect, a hybrid system, incorporating best practices from both the EU and the United States NSSP (chapter 13).

14.1.1 Responsible authority

In Scotland, the responsible authority for bivalve shellfish regulatory control is the Food Standards Agency Scotland (FSAS). Although the FSAS is a Government agency, it does not report to a specific minister and is free to publish any advice it issues in the form of guidance notes and advisory circulars. The FSAS delegates responsibility for some official controls to the Local Food Authority. Thus enforcement of the provisions is undertaken via a combination of central and local government, but the FSA retains overall accountability to the European Commission. In Canada, the Canadian Shellfish Sanitation Programme (CSSP) is jointly administered by three federal bodies. In New Zealand, the New Zealand Food Safety Authority (NZFSA) was established as a Public Service department on 1 July 2007 and reports directly to a government minister. Thus, these three countries have different reporting systems.

14.1.2 Shellfish production quantum

The pressures are different on the three countries discussed. In Scotland there are 186 classified shellfish harvesting production areas comprising 246 individual

harvesting sites. Six main species of bivalve mollusc are harvested, common mussels and Pacific Oysters dominating the aquaculture trade, clams and cockles the wild shellfisheries, a total of around 4000 tonnes with a value of around US$10M. In New Zealand there are 87 classified shellfish growing areas, most of these being aquaculture sites. Approximately 108 000 tonnes of bivalve molluscs are produced annually in New Zealand, 97 000 tonnes of which are Greenshell$^{®}$ mussels, *Perla canaliculus,* produced exclusively via aquaculture (2006 figures – total value US$200M). Around 80% of the bivalve molluscs produced in New Zealand are exported to more than 60 countries worldwide. Canada has more than 1000 classified shellfish production areas with a combined coverage in excess of 21 000 km^2. In the year 2005, Canada produced US$146M worth of wild harvested bivalve molluscs, some 89 000 tonnes (predominantly scallops and clams). In that same year, the production and value of cultivated bivalve molluscs was around 38 600 tonnes (predominantly mussels and scallops) with an estimated value of approximately US$67M.

14.1.3 Categories of shellfish waters

In terms of classification outcomes, Scotland recognizes three categories of shellfish waters based on *E. coli* levels, namely: category A, where products can be put directly to market, category B and C where the product must go through either depuration, designated heat treatment or relaying prior to reaching the market place. These are standard EU categories. In New Zealand and Canada the United States approach prevails and defines: "remote approved", "approved", "conditionally approved", "restricted" and "conditionally restricted" areas. The rationale behind the conditional and restricted categories is exactly the same in the three country systems – closures are applied on a restorable basis depending on factors that affect the shellfish and/or the shellfish water quality.

14.1.4 Sanitary surveys and annual review

As to be expected, Canada, Scotland and New Zealand all underpin their bivalve shellfish regulatory regimes with extensive sanitary surveys. Although the terminology applied may differ, all three systems utilize effectively the same methods and data sources. In addition, all undertake annual reviews of approved areas; for example the Canadians apply a rigorous, comprehensive survey based on 15 sampling runs performed at random dates over a calendar year. In addition, there are annual reviews of approved areas plus a complete re-evaluation of each approved site at least triennially. Scotland is in the throes of setting up sanitary survey reports for all existing and new fisheries which will

take the final form of a sampling plan for the area. Annual review also features highly in Scotland's plans. In these, a minimum of nine samples is required, taken in separate months, between January and December to maintain an area classification. The NZFSA consider the risk assessment approach, which is a sanitary survey, to be a critical component in establishing the evidence base for shellfish safety. For potential new growing areas a minimum of 30 water and 30 shellfish flesh samples are required to be analysed from samples reflecting a variety of environmental conditions over a minimum 12-month period. They too produce an annual review of the sanitary survey and include the bacteriological data taken under adverse environmental conditions.

14.1.5 Monitoring programmes

The monitoring programme in Canada is immense – a 21 000 km area of harvest waters translate into more than 1500 sampling stations. That notwithstanding, it is also the case that the majority of Canadian waters are currently not monitored due to factors as variable as resource availability, distance and climate. As stated previously, the CSSP basically applies the United States Food and Drug Administration (US FDA) bacteriological parameters comprising faecal coliforms in shellfish waters but not in the shellfish flesh. In Scotland, the EU legislation is strictly adhered to requiring determination of *E. coli* levels in shellfish flesh. In New Zealand, the drive to minimize closures to ensure security of supply to 64 export partners lead in 2004 to an overhaul in its procedures to ensure consistency in dealing with so many partners. Since the 1970s, New Zealand had based its regulatory approach on US FDA protocols which are still evident in the current system, but this has now included some EU practice modified by the particular New Zealand perspective. Thus, in effect both the US FDA shellfish waters and the EU shellfish flesh protocols apply. Analytical methods were varied slightly to reflect those favoured in Europe. The most difficult questions were what level of *E. coli* to apply to the shellfish flesh and should levels in the flesh apply to the classification as well as the NSSP water quality levels or in support of them? It was decided that *E. coli* levels in shellfish should be used for classification purposes and that the same median and percentile approach as used for water quality should be used for shellfish quality – a sort of belt and braces approach.

14.1.6 Other aspects – communication, mitigation and liaison with stakeholders

Examination of chapters 11, 12 and 13 clearly indicate that, for virtually all aspects of the regulatory regime, each country takes appropriate steps to

engage with the relevant stakeholders. For example, public shellfish harvest areas are subject to public notices and press campaigns if any harvesting restrictions apply. Although far from foolproof, this approach demonstrates a clear intent to alert casual collectors to the existence of real hazard conditions. In the same way there are well-documented consultation processes, clear liaison with industry, local, regional and international collaborations. Where triggers are exceeded, clear actions that are to be taken are identified in all countries.

Not all the actions are identical, not all the processes follow the identical set of pathways, nor would they be expected to with different approaches to the central pillar of monitoring between the countries. Adopting a consistent approach to monitoring would help smooth out problems oscillating around complementarities of data, but that is for discussion in chapter 17.

14.2 A SURVEY OF CURRENTLY ACHIEVED MANAGEMENT PRACTICE IN SELECTED LOCATIONS

The approaches towards microbiological monitoring of shellfish for the presence of pathogens/indicators vary in different countries, largely determined by capacity. Resource rich developed regions and countries such as the EU and the United States have systems where either shellfish or harvesting waters are monitored. In these cases the EU or US FDA methodologies are the two accepted predominant management approaches (Lees 2000). In other countries, depending on resources, capacity and other factors, a spectrum of management responses exist. The subsequent sections will go on to examine some of those responses to provide a more complete perspective on achievable management strategies for bivalve fisheries around the world. The examples will outline a well articulated but poorly implemented system in an expanding economy (China), a regional perspective from the Mediterranean and the situation in a country with a well-developed science and research base that apparently lacks a clearly defined system (India).

14.2.1 Controls on cultivated bivalves in China

China produces about 11 million tonnes of bivalves annually through commercial aquaculture programmes (China Fishery Statistical Yearbook 2006), which is about 70% of total world production, and 95% of these products are consumed domestically.

China has made great efforts in the past decades, developing a series of regulations and national standards to control the safety of aquatic products. However, the lack of systematic management and effective control procedures over the entire process of bivalve production is a major problem for Chinese administrators and producers. For example, there is a lack of consistent monitoring programmes for chemical residues and other pollutants in bivalves, unclear stipulations on the responsibilities of different stakeholders, such as the responsibilities for site opening/closure and producer responsibilities for food safety events, and an incomplete early warning and recall system. Additionally, harvesting area classification is still an ongoing project.

14.2.1.1 Legislative mechanisms

More than 20 major national standards have been issued during the past 11 years, either governing the hygiene of the products or prescribing water quality criteria and monitoring methods, and these include:

- GB2744-96 – Hygiene Standard for Marine Bivalves;
- GB16324-1996 – Hygiene Standard for Dried Marine Bivalve Products;
- GB 2742-1994 – Hygiene Standard for Oysters;
- GB/T4789.1, 2, 3, 4, 5, 7, 10-2003 – General Principles for Foodstuff Microbiological Test and Foodstuff Microbiological Test series, including total bacterial colony counts, *Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus*, and pathogenic staphylococci;
- GB/T 5009. 1-2003-GB/T 5009. 203-2003 – Hygiene Test for Foodstuff;
- GB3097-1997 Sea Water Quality Standard;
- GB11607-1989 Water Quality Standard for Fisheries;
- GB12763.4-91 The Specification for Oceanographic Observations of Chemical Parameters in Sea Water;
- GB12763.6-91 The Specification for Oceanographic Observations of Biological Parameters in Sea Water; and
- GB 17378-1998 The Specification for Marine Monitoring (series 1–7).
- Note: GB denotes 'National Standard of the People's Republic of China'.

14.2.1.2 Monitoring protocols

Microbiological monitoring of Chinese shellfish waters is undertaken using thermotolerant or faecal coliforms as determinants. Monitoring is also undertaken for biotoxins including Diarrhetic Shellfish Poison and Paralytic Shellfish

Poison and heavy metals such as Cu, Cr, Hg but these do not feature in this monograph.

For the assessment of microbiological water quality, total and faecal coliforms are measured using either multiple tube fermentation or membrane filtration methods according to the relevant national standards (i.e. GB17378-1998). The indicator levels are assessed against the following levels: total coliforms ≤500 /L, faecal coliforms ≤140 /L.

Monitoring of the actual shellfish product, the flesh, is undertaken with a similar range of parameters. For the microbiological components these include total counts of colony forming units (cfu), total coliforms, *Salmonella* spp., *Shigella* spp., *V. parahaemolyticus* and pathogenic staphylococci, all determined by the most recent editions of national standards for testing shellfish: total counts, GB/T4789.2-2003; coliform group, GB/T4789.3-2003; *Salmonella* spp., GB/T4789.4-2003; *Shigella* spp. GB/T4789.5-2003; *V. parahaemolyticus*, GB/T4789.7-2003; pathogenic staphylococci, GB/T4789.10-2003. In terms of compliance, total bacterial counts in bivalve flesh must not exceed 10^5/g; total coliforms 300 MPN/100 g and there must be zero *Salmonella* spp. in 25 g flesh.

14.2.1.3 Requirements for depuration and dispatch centres

Requirements for these processes include a range of factors, including tap water, depths of drains, flow of water, and sanitary conditions.

Purification centres must have their own laboratories or be able to access a laboratory equipped with necessary facilities, so that the effectiveness of the purification process can be determined through microbiological tests. Purification centres must record data regularly, including the source of the live bivalves and their quality, harvesting date, duration of purification and relaying, the health condition after purification, dispatch details or consignment after purification.

14.2.1.4 Implementing the legislation

Samples of live or processed bivalve products must be tested before sale. Depuration is recommended for live samples that could not meet the official standards and farming areas will be closed (for an appropriate period) if biotoxin levels are above the limits, or products on sale withdrawn if biotoxin or other safety indices are above the regulatory limits. An official report is produced after testing each sample and sent by mail or fax to the farmers or processors who are then responsible for taking any remediation measures. Serious violations or actual harm done to human health will incur penalties and may result in withdrawal of the license (or permit for production) as detailed in the Temporary Regulation on Monitoring and Management for Hygiene of the Bivalve

Cultivation Environment (TRMMHBCE) enacted by the Supervision and Management Bureau of Fishing Administration and Fishing Harbour, Ministry of Agriculture of China (1997).

14.2.1.5 Responsible authority

Monitoring the environmental and sanitary quality of shellfish waters is undertaken by local fisheries' environmental monitoring stations, according to GB3097-1997. The Local Oceanic and Fisheries Bureau is the competent authority responsible for enforcing the law. Quality and safety of bivalve products on the market are supervised by the local Health Bureau or Bureau of Quality and Technical Supervision (for domestic commodities) and entry–exit Inspection and Quarantine Bureau (for export commodities). The latter two are under the supervision of the General Administration of Quality Supervision, Inspection and Quarantine of People's Republic of China (AQSIQ).

There is a complexity of management systems in China for the quality of aquatic products. Different levels of Oceanic and Fisheries Bureaus report to the Bureau of Fisheries of the Ministry of Agriculture for management of aquaculture activities, while they also report to the State Oceanic Administration for issues such as sea area allocation and the issuance of utilization licenses. As for the marketing of fish products, both the local Health Bureaus and Bureau of Quality and Technical Supervision have the right to demand a withdrawal of unhealthy products (more usually a confiscation in this case) and closure of relevant fishing/cultivation areas for a certain period of time. However, for export to third countries, the different levels of entry–exit Inspection and Quarantine Bureaus are in charge of the inspection and testing.

Although not law enforcement bodies themselves, companies such as Intertek Testing Services and SGS-CSTC Standards Technical Services Co., Ltd. are often commissioned to provide the third party accreditation for aquatic products being exported to foreign countries. Their work has become an integral part of quality control system in China, and their test results enjoy an adequate legal status.

14.2.1.6 Implications of non-compliance

At present, an oral or written warning is the usual measure to be taken when non-compliance occurs. Subsequently TRMMHBCE can impose further penalties, including:

- For Class Three waters (see 14.2.1.7 for details of shellfish water classifications), if it is difficult to improve the water quality in a short time, or in case of long-term pollution, the fisheries management

authority (local Fisheries Bureau) should close the relevant farm, or ban the on-going shellfish cultivation activity, and forbid harvesting.

- For areas that are temporarily classified as Class Three waters due to occasional pollution incidents, including red tides, temporary closure will be implemented. The fisheries authority will demand that the monitoring stations follow the pollution incident closely, allowing for re-opening of the farms only when the pollution disappears and no residue of pollutants and biotoxins are detected.
- No harvesting of bivalves is permitted in temporarily closed areas. Cultivating or harvesting bivalves in long-term closed Class Three waters, and collecting bivalves in temporarily closed areas without permission are illegal, and the collected products will be destroyed.
- For those bivalves collected in temporarily closed areas without permission which had poisoned consumers after consumption of the products, the activity of harvesting and sale will be banned immediately; the products will be destroyed, the illegal income confiscated, and a fine of one to five times of the illegal income will be imposed. If no illegal income has yet been generated, a fine of between 1000 and 50 000 yuan will be imposed (US$140–6900). For those who have caused severe harm to consumers' health, further liabilities and penalties are likely.
- Institutions and individuals who engage in bivalve purification and relaying should meet with hygiene requirements and produce shellfish that is up to food safety standards; upon violations, a warning for correction will be in place, and a fine of up to 5000 yuan (US$690) will be imposed; failure to correct or other serious violations will result in the withdrawal of the sanitary license.
- Institutions and individuals who discharge wastes into the shellfish cultivation areas and bring about environmental pollution or bivalve contamination, will be accused according to Item 42 of Marine Environment Protection Law of the People's Republic of China (effective as of 2000).

14.2.1.7 Assessment for classification of harvesting areas – legislative backdrop

The TRMMHBCE is currently the most complete regulation in China governing bivalve production. This regulation stipulates the hygiene requirements for bivalve cultivation (including cultivating and harvesting areas), depuration, relaying and dispatching. For example, defining the competent authority

(hierarchical Fisheries Management Authority), licensing of cultivation estab-lishments, cultivation area classification into three categories, enforcement procedures (closure and re-opening of farms in Class Three water areas), and monitoring regimes is clearly defined or listed in items 3–7. There is also a preliminary requirement on traceability of bivalve products (items 9 and 15).

As listed in TRMMHBCE, bivalve shellfish production areas can be classified into three types, based on results of water quality and the sanitary quality of bivalve products (correlating with the US FDA rather than the EU approach):

(1) *Class One*: Water environmental quality and bivalve sanitary quality accord with relevant national standards. The shellfish cultivated or caught in this area can be put on the market directly for human consumption.

(2) *Class Two*: Water environment is slightly polluted and levels of some pollutants exceed the regulated standards in shellfish meat. But the bivalves produced in this area can meet relevant national standards for sanitary quality after purification or relaying. The bivalves cultivated or collected in this area can be put on the market for sale after purification or relaying.

(3) *Class Three*: The water environment and the shellfish are seriously polluted, and the bivalves produced in this area are unable to meet relevant national sanitary standards with available treatment measures. The bivalves thus produced are forbidden for human consumption.

In addition, there is also a draft of Shellfish Harvesting Area Classification compiled by researchers in the Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (YSFRI). In this draft standard, the criteria for harvesting area classification are based on the purpose and hygiene requirements of bivalve production. The classification criteria and details are listed in Table 14.1. To date, this is unpublished.

An on-going Harvesting Area Classification project (2007) is governed by the Fisheries Bureau of Ministry of Agriculture. Shellfish waters are classified into three categories according to microbiological index (number of *E. coli*) following the guidance of GB4789.3-2003 – Test of Coliforms in Foodstuff.

• Class One: number of *E. coli* < 300/100 g meat, bivalves harvested in this area can be put to market directly and consumed without cooking;

• Class Two: *E. coli* 300–6000/100 g meat, bivalves harvested in this area can be put to market directly with advice to consume after cooking; and

- Class Three: *E. coli* >6000/100 g meat, bivalves harvested in this area can be put to market after purification, reducing the number of *E. coli* to Class Two levels.

Table 14.1 Draft standards for harvesting area classification in China

Items	Class One	Class Two	Class Three
Floating materials	No oil film, foam, and other floating materials on the water surface		Oil film, foam, and other floating materials on the water surface
Colour, smell, and taste	No abnormal colour, smell, and taste in the seawater		Abnormal colour, smell, and taste in the seawater
Suspended materials (mg/L)	Those added by human activities ≤10		Those added by human activities >10
Faecal coliforms/L	≤700	≤10 000	>10 000
E. coli/L	≤140	≤2000	>2000
Pathogen	No pathogens		Some pathogens

Source: Yellow Sea Fisheries Research Institute, draft working standard, 2004

14.2.1.8 Sampling regimes

According to item 3 in the Annex of TRMMHBCE, the water quality of the cultivation areas and shellfish hygiene indices should be tested monthly from May to October and bimonthly from November to April. During times of harmful algal bloom occurrence, testing should be done daily. However, in practice, this rule is not followed closely. Some cultivation areas are rarely monitored; other areas may be sampled and tested only four times per year. However, it is intended that in the future the standard is enforced and the sampling frequency will be on a regular monthly basis.

14.2.1.9 Local competent authorities and licensing of "depuration and dispatch centres"

According to TRMMHBCE, the local competent authority should be the hierarchical Fisheries Management Authorities. These are the provincial and municipal Oceanic and Fisheries Bureaus, which are the integrated subordinates of the State Oceanic Bureau and Fisheries Bureau of the Ministry of Agriculture of China.

Although a complex process, it is generally agreed that the Oceanic and Fisheries Bureaus of different levels are in charge of regulating the process, especially the division, allocation and licensing of aquaculture areas. However,

criteria such as the Harvesting Area Classification have not been consistently enforced in many cultivation areas so far.

14.2.1.10 Current infrastructure – reference and monitoring laboratories

There are two national reference laboratories for the testing of aquatic products, including the National Centre for Quality Supervision and Test of Aquatic Products, which is affiliated with the YSFRI. The output of these laboratories is collated on an annual basis.

Microbiological, biotoxins and other contaminants are routinely tested for in foodstuffs in a hierarchy of national, provincial and municipal level Health Bureaux (supervised by the Ministry of Health), and Bureaux of Quality and Technical Supervision and entry–exit Inspection and Quarantine Bureaux (under the supervision of AQSIQ). In addition, there are approximately 18 food quality laboratories affiliated with the Ministry of Agriculture that test for micro-biological indices and other contaminants in agricultural products, including aquatic products.

The eligibility rate of Chinese export foodstuff is higher than 99%. In addition to legislation and enforcement improvements, such as implementing the Harvesting Water Classifications, the Government of China has also been working on marine environmental protection and remediation. Pollution control programmes have been carried out in major sea cultivation areas, and special environmental monitoring institutes like the Centre for Supervision and Test of Fishery Ecological Environment in the Yellow/Bohai Seas (MOA) have been set up, which has the legal right of producing testimony in lawsuits concerning pollution of shellfish cultivation waters.

14.2.1.11 Summary of the situation in China

There is a legislative system safeguarding the food quality of cultivated bivalves in China. An overall regulation (TRMMHBCE) was enacted in 1997, which deals with the overall procedures from cultivation to placing on the market of bivalves in China. However some revisions would help meet the needs of the industry of today. Relevant national standards are also used for operational guidance.

There are also a whole set of regulations supervising the processing of fish products. Since most bivalve products on domestic markets are sold alive or without much processing, these regulations are generally applied for export, and are duly enforced by the hierarchical entry–exit Inspection and Quarantine

Bureaux. These regulations always embrace the same standards and 'test limits' of the destination countries, by emphasizing the establishment of accredited management regimes (such as HACCP and good practices), and generally up to EU and US FDA standards. It is a legal requirement that all the processors obtain a licence from their local entry–exit Inspection and Quarantine Bureaux and receive routine inspections and those who export to third countries must obtain EU or US FDA certificates. The entry–exit Inspection and Quarantine Bureaux checks the performance of all the licensed processors monthly and official examinations under the supervision of a jury is held biannually.

However, the current Chinese legislation and enforcement regime is rather different from the requirements of US FDA or EU directives governing the quality and safety, and marketing of cultivated bivalve products. Small companies and farmers in China are usually not effectively supervised and monitored, due to the lack of resources for internal quality control and a somewhat fragmented supervisory system administered by the local food authorities. However, larger companies, particularly those involved in processing, are generally well controlled by internal as well as external (governmental) regulations. Indeed, one thing noteworthy for these larger companies is that most of them have introduced US FDA or EU standards under their own initiative and have already obtained US FDA certificates for export of their products. For example, about 90% of processors in Shandong Province have obtained FDA certificates, of whom FDA takes a selective check annually. In addition about 50–60% of processors in Shandong have obtained EU certificates, of which the EU may take a selective check at any time.

14.2.2 Mediterranean states

This section draws very heavily and often directly on a recent report produced by United Nations Environment Programme (UNEP) Mediterranean Action Plan – Assessment of the State of Microbial Pollution of the Mediterranean Sea, published in June 2007. This document went through a separate process of peer review and we are indebted to Dr George Kamizoulis, Senior Scientist, WHO/EURO Project Office Coordinating Unit for the Mediterranean Action Plan for making the report and its output fully available to us.

As a general rule, in Mediterranean countries, individual growing areas are classified according to European standards for those countries belonging to the EU. The rest of the Mediterranean countries follow their own national guidelines or the European standards or appear to have no policies or processes. Some potentially productive growing areas remain prohibited for harvest because of inadequate state resources to conduct the requisite sanitary surveys.

The key features of national legislation and related measures concerning the quality of shellfish waters and shellfish in the various Mediterranean countries are summarized in the following paragraphs. Unfortunately, the picture is not complete, as information from some countries was not readily available to the report's authors.

14.2.2.1 Mediterranean states that are also EU member states

Seven nations fall in to this category – France, Spain, Italy, Greece, Slovenia, Cyprus and Malta. The first four countries all regulate their shellfish industries based on the Water Framework Directive (2000/60/EC) and the European Food Standard Regulations 854/2004.

There may also be old national practices that continue. For instance France still operates an administrative standard for shellfish growing waters (that has no statutory standing) into four categories as follows:

A: Satisfactory, 0 *E. coli* per 100 ml seawater
B: Acceptable, 1–60 *E. coli* per 100 ml seawater
C: Doubtful, 61–120 *E. coli* per 100 ml seawater
D: Unsatisfactory >120 *E. coli* per 100 ml seawater

In Greece, apart from the guide standard of 300/100 ml flesh and intervalvular fluid for faecal coliforms, the law also sets a mandatory standard of 700/100 ml. Shellfish satisfying the guide value are acceptable for human consumption, those satisfying the mandatory value are subjected to depuration. The microbiological quality of shellfish produced in small quantities for the Greek local market (up to 100 kg per day) is governed by health regulations stipulating that shellfish sampled in the market should not exceed 5 faecal coliforms/ml of flesh to be considered suitable for consumption. Shellfish containing between 6 and 16 faecal coliforms/ml of flesh require depuration before consumption, while those containing more than 16/ml are considered unsuitable for consumption. There are currently 24 officially monitored shellfish productions zones in Greece.

In Spain depurated shellfish destined for consumption must comply with the following microbiological standards:

- Aerobic microorganisms: up to 100 000/g
- *Escherichia coli* up to 500 per litre flesh and intervalvular fluid
- *Salmonella* spp. absent in 25 ml flesh and intervalvular fluid
- Streptococci (Group D) up to 100/g
- *Vibrio parahaemolyticus* up to 100/g

Italy follows the EU guidelines (854/2004) for shellfish. Shellfish-growing waters are still classified into approved zones and conditioned zones, with the following standards:

- *Approved zones*: Seawater should not contain more than two *E. coli* per 100 ml. Up to 7 per 100 ml seawater is tolerated in not more than 10% of the samples, provided that the shellfish themselves come up to the required standards. Shellfish should not contain more than four *E. coli* per ml of flesh plus intervalvular fluid, and *Salmonella* spp. must be absent in 25 ml flesh plus intervalvular fluid.
- *Conditioned zones*: Seawater should not contain more than 34 *E. coli* per 100 ml. Up to 49 per 100 ml are tolerated in not more than 10% of the samples. Shellfish should not contain more then 39 *E. coli* per ml of flesh plus intervalvular fluid.

Depurable species are only cleared for direct consumption if they originate from culture areas in an approved zone. Depurable species originating from (a) natural breeding grounds and (b) culture areas in conditioned zones are subject to mandatory depuration prior to consumption. Those originating from natural breeding grounds in conditioned zones must be cooked prior to consumption. Non-depurable species are cleared for direct consumption if they originate from approved zones, or from culture areas in conditioned zones, otherwise they are subject to mandatory cooking. Under Italian Law, Class A zones also have a requirement for *Salmonella* spp. and *Vibrio* spp. (0 in 25 g flesh plus intravalvular fluid). Stabilization zones are also included, with the same standards as for Class A zones.

In Slovenia, water quality control processes for shellfish breeding are contained in the 1988 Slovenian Decree on Preventive Vaccination, Diagnostics and Research in the Relevant Field. The standard for acceptable shellfish waters is 10 faecal coliforms/100 ml flesh, based on a fortnightly sampling frequency.

There are no legal standards for shellfish water quality in either Cyprus or Malta. In Malta public health legislation deals with shellfish for consumption and no shellfish products can be sold unless the trader is holding a permit issued by the Superintendent of Public Health. As of June 2007 there were no valid permits issued in Malta for the sale of fresh shellfish, although there was activity governing shellfish imported for human consumption. There are no officially designated shellfish growing areas in Cyprus. Any shellfish harvested originate from unofficial shellfish growing areas and are consumed locally.

14.2.2.2 Other Mediterranean states

Algeria

There is no information for official shellfish growing areas or regulation and control procedures running in Algeria.

Bosnia and Herzegovina

Standards for shellfish growing waters in Bosnia are based on EU standards, however no effective surveillance programme exists.

Croatia

In Croatia, shellfish water is classified in the articles of the Water Classification Decree of 1981, where four classes of coastal sea water are delineated based on both microbiological and physicochemical parameters. In terms of microbiological standards, the Croatian standard deems as acceptable a concentration of total coliforms of not greater than 100 cfu/100 ml for shellfish harvest waters.

Egypt

There are no specific statutory standards or criteria under Egyptian law regarding the microbiological quality of shellfish waters or shellfish flesh. However, shellfish waters are examined at regular annual intervals. These data are then apparently evaluated according to international (global) and European standards. Enforcement is through internal administrative procedures emanating from the Ministry of Agriculture as lead Government department. However, there is no official monitoring programme as most of the shellfish are consumed locally.

Israel

As shellfish are not grown or harvested in Israel, there are no requirements for any related standards.

Lebanon

There is no information available on shellfish harvesting, production or monitoring for Lebanon.

The Libyan Arab Jamahiriya

Apparently, there are no national standards currently in force for shellfish waters in the Libyan Arab Jamahiriya. However, pending the development and adoption of new standards, which are currently being finalized, the Libyan Arab

Jamahiriya is observing the standards adopted by the Contracting Parties of the Mediterranean Action Plan in 1987.

Monaco

There are no official shellfish growing areas along the Monaco coastline.

Montenegro

No data is available on shellfish waters or monitoring in Montenegro.

Morocco

Microbiological quality standards and criteria for shellfish waters in Morocco are based on French (and therefore EU) legislation. There is, however, little information readily available on the implementation of that legislation or any monitoring programmes.

The Syrian Arab Republic

No data is available on shellfish waters or monitoring in the Syrian Arab Republic.

Tunisia

Tunisia has 16 traditional shellfish growing waters classified into three categories:

- Sanitary zones: Shellfish flesh up to 300 faecal coliforms per 100 ml; *Salmonella* spp. absent in 25 g; water up to 2 faecal coliforms per 100 ml.
- Conditioned zones: Shellfish flesh up to 3900 faecal coliforms per 100 ml; water up to 34 faecal coliforms per 100 ml.
- Unsanitary zones: Shellfish flesh above 3900 faecal coliforms per 100 ml; water above 34 faecal coliforms per 100 ml.

Whenever a site is found to be contaminated, it is closed and then re-opened again when the water quality and/or shellfish flesh improves and meets acceptable conditions.

Turkey

The Aquatic Products Law came into force in Turkey in 1971, was amended in 1995, and contains general conditions and regulations for coastal protection and production of aquatic products. This law regulates the discharges to fish and shellfish production areas and sets acceptable values in receiving waters.

The Ministry of Health is responsible for the coordination of activities related to aquatic products at both national and international levels.

The Turkish Quality Control System for fishery products was introduced in 1998 and has been developed under the Fishery Law, the Fishery products regulations and EU Directives (91/493/EEC, 91/492/EEC, 79/223/EEC and 94/356/EEC) and the FAO Standard (Codex Alimentarius). There are two classes and four regions in Turkey (1999/767/EC Decision); Two in A class (live bivalves and molluscs-91/492/EEC) and two in B class. The harvesting season generally runs from 1 September to 1 May. The difficulties in applying a monitoring programme include the lack of a clear limit for some parameters and consistency of sampling in bad weather conditions. The annex to the regulations defines limits on activities and substances. The microbiological limits for harvesting waters are:

- total coliforms not to exceed 70 per 100 ml;
- faecal coliforms not to exceed 10 per 100 ml; and
- *E. coli* not to exceed 2 per 100 ml (extendable to 7 per 100 ml).

14.2.3 India – a system under development

Shellfish culture is not yet effectively organized on a commercial basis in India and most of the shellfish consumed is harvested from natural beds and consumed locally. The Indian Marine Products Export Development Authority (MPEDA) has initiated a monitoring programme wherein mussels are monitored for faecal coliform counts and presence of biotoxins, but its extent and regulatory worth is unknown. This is surprising for a country with such an active academic community and a responsible approach to regulatory regimes.

Surveillance and monitoring notwithstanding, research into microbial contamination of shellfish populations in India has taken place and is continuing. Deepanjali *et al.* (2004) conducted a two-year study on the prevalence of pathogens, *V. parahaemolyticus*, *V. vulnificus,* choleragenic *V. cholerae* and of indicator bacteria (faecal coliforms) in oysters from two estuaries along the coast of Karnataka. In addition, presence of enteric viruses and coliphages were also monitored. This study focussed on two estuaries close to Mangalore -. Mulki and Sasthan. There was no correlation between the levels/presence of tdh[+] *V. parahaemolyticus* and presence/levels of faecal coliforms. Faecal coliforms were detected in all shellfish samples while tdh[+] *V. parahaemolyticus* was observed in only 10.2% of samples. Choleragenic *V. cholerae* was not detected in oysters during the two year study. This suggests that *V. cholerae* O1/O139 may not be common in the environment in this region.

Another important human pathogen that is likely to be associated with shellfish is *Salmonella* spp. Studies with clams from markets in India indicate that 20% are positive for *Salmonella* spp. (Kumar *et al.* 2003). However, in tropical waters rich in organic matter, *E. coli* and *Salmonella* spp. could persist for a long time and even multiply (Winfield and Groisman 2003). Studies in India show that most of the seafood associated *Salmonella* spp. infections belong to the serotype *S. weltevreden* (Kumar *et al.* 2003). However, in the United States, *S. weltevreden* is rarely found in human cases. In contrast, studies in Thailand reveal that *S. enterica weltevreden* is the most common serovar associated with human Salmonellosis (Bangtrakulnonth *et al.* 2004). Thus the clinical significance of *Salmonella* serotypes isolated from aquatic environments need to be studied further.

These findings, albeit quite rudimentary, suggest that the introduction of a monitoring programme in India would help to to protect consumers from risks associated with consuming contaminated bivalves.

14.3 CONCLUSIONS

This chapter is a brief illustration on the inconsistent approach to the management of bivalve shellfish destined for human consumption. It confirms that resources and commercial circumstances dictate the nature and extent of the regulatory response. Thus, we have a significant number of well-resourced countries where there is no appreciable management response to the issue because the shellfish are either not harvested or generally harvested and consumed locally. The commercial imperatives appear to come in two broad categories. First, internal markets within a country will lead to some resource allocation and some control processes to safeguard domestic consumers. Second, if the shellfish are to be exported to other countries, then there will likely be a much greater allocation of resources required as there will likely be far more comprehensive controls to satisfy. The existence of lucrative export markets, for instance for the New Zealand shellfish industry, brings with it financial rewards but also the requirements to meet exacting food safety requirements for the export customers. Thus it is predominantly the commercial demand for the shellfish that dictates the allocation of resources for monitoring and control. Where there are sufficient resources, there is still limited consistency and no real attempt to harmonize approaches unless there is a real commercial imperative such as meeting the demands of different export markets (as in New Zealand, see chapter 13).

Within a naturally defined region such as the Mediterranean, practice varies enormously on a scale from no management through to full, careful

implementation of EU legislation. Then we have countries such as China and India on remarkable economic development trajectories who either have a wonderfully complex but poorly implemented set of management controls (China) or have at best an understanding of the issue that is largely ignored (India). In effect, the Chinese response can be seen to clearly demonstrate the paradox created when the export markets demand an exacting regulatory set of controls and get them, thus making the associated costs an essential part of the business. The internal market within China largely avoids the same levels of control as they are seen as an additional and unwelcome cost to internal consumers.

The system needs harmonizing and re-structuring in such a way that countries can proceed along a pathway that increasingly develops their capacity to accurately monitor health risks. Whilst the logic of responding to the market place is clear, all consumers deserve the same degree of protection. Thus, the casual collectors and those they supply should be as safe from infectious disease as are the export customers. Applying fairly unsophisticated sanitary surveys alone, right up to monitoring on a regular basis for the right indicator in the right milieu – flesh or water – using best available analytical techniques, all on a consistent basis, should be a common goal.

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15

Experience from recreational waters

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Management of recreational waters is changing rapidly to accommodate recommendations driven by World Health Organization (WHO) (WHO 2003). Parallel developments in drinking water quality management have also been evident (WHO 2004). These water quality Guidelines establish the concepts of 'profiling' of recreational water and 'water safety planning' for drinking water, both based on hazard analysis and critical control point (HACCP) principles. The parallel development of real time prediction of adverse water quality conditions to facilitate the control of public health risks through the implementation of an appropriate management system presents identical challenges and opportunities in both recreational and shellfish harvesting waters. This chapter outlines some lessons from studies designed to support implementation of the WHO approach to recreational water management and suggests key research and management questions in the risk assessment debate for both water types.
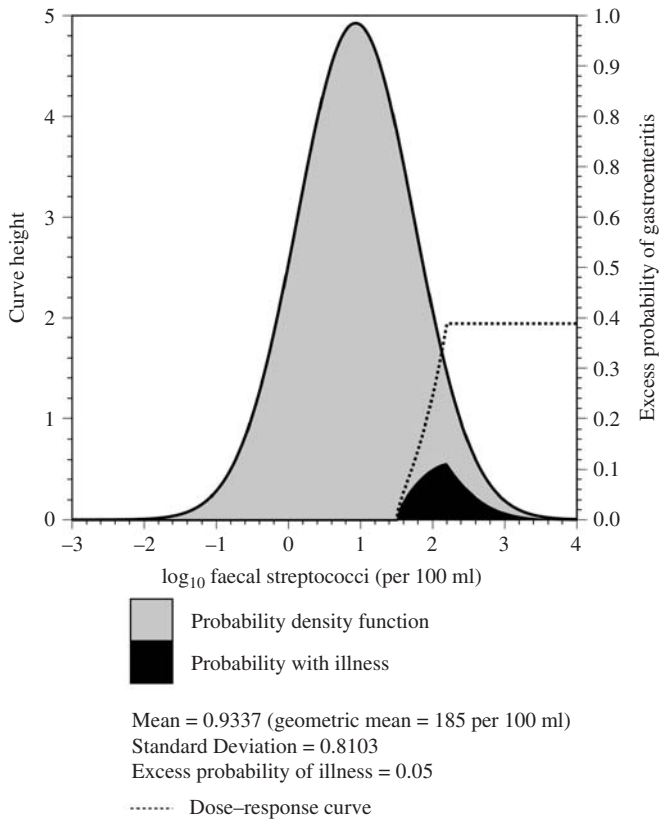
15.1 GUIDELINES DEVELOPMENT

The WHO Guidelines for Safe Recreational Water Environments (GSRWE) (WHO 2003) outline a radical new paradigm in environmental regulation which presents both a challenge and opportunity to the regulatory community. Stated briefly, the GSRWE develops two guiding principles of recreational water management. The first is that numerical microbiological standards should be based on epidemiological evidence of health risk and the second is that the Guidelines should be sensitive to the environmental processes and variability in the main parameters used to measure 'compliance' at bathing waters – the faecal indicator organisms (FIOs).

To assess the evidence on which to base the GSRWE, the WHO undertook an internal review of the international epidemiological literature (Prüss 1998). The objective was not to produce a meta-analysis of the health evidence base relevant to recreational water exposures but, rather, to facilitate selection of the most appropriate protocols and dose–response relationships for use in the standards design process. This process resulted in the formulation of numerical standards published as a WHO Consultation Draft in 1998 (WHO 1998).

The mathematical derivation of the risk-based standards utilized a new approach which combined a probabilistic measure of exposure, in the form of a probability density function for intestinal enterococci concentration in the recreational waters, with a dose–response relationship giving a continuous risk assessment in terms of the probability of a bather acquiring gastroenteritis from one bathing exposure (Kay *et al.* 1994; Fleisher *et al.* 1996; Kay *et al.* 2004). Box 1 illustrates this procedure for a 'theoretical' recreational water. Clearly, exact risk assessment would require both the geometric mean faecal indicator concentration and its $\log_{10}$ standard deviation for any site (assuming $\log_{10}$ normality for the FIO predicting illness, intestinal enterococci). An earlier set of water quality criteria, developed for the States of Jersey, utilized both distributional parameters (Wyer *et al.* 1999). However, this was felt to be too complex for international implementation and the WHO team of independent technical advisers suggested a single parameter, the 95th percentile, should be used in the published guidelines (Kay *et al.* 2004).

The numerical criteria published in the WHO Draft Consultation (WHO 1998) were more stringent than the mandatory standards in force in Europe and North America and it was clear to the scientific community that simple implementation of the numerical standards would result in very significant increases in beach failures world-wide. Furthermore, examples were cited of locations only marginally affected by human sewage discharges but which would still fail the proposed numerical guidelines due to 'normal' variability in

Box 1 Epidemiological dose–response relationship and probability density function to derive water quality guidelines from epidemiological results. Source: Kay *et al.* (2005).

microbiological concentrations derived principally from diffuse source agricultural pollution generated by livestock enterprises (Faust 1976; Niemi and Niemi 1991; Wyer *et al.* 1994; Wyer *et al.* 1996; Wyer *et al.* 1997; Wyer *et al.* 1998; Jagals *et al.* 1995; Cook *et al.* 1996; Aitken *et al.* 2001; D'Arcy and Frost 2001; Anon 2002, 2003, 2004; Shreeram and Mostaghimi 2002; Vinten *et al.* 2002; Vinten *et al.* 2004a; Vinten *et al.* 2004b; Avery *et al.* 2004; Benedict and Neumann 2004; Davies-Colley *et al.* 2004; Deeks *et al.* 2005; Kay *et al.* 2007).

Detailed consideration of this aspect was undertaken at a further expert consultation in Annapolis, USA which led to the 'Annapolis Protocol' (WHO 1999). This established the process of sanitary inspection which has become

known as 'beach profiling' and the linked process of risk management through real-time prediction of adverse water quality and the provision of timely public information through the provision of advisory notices. At the core of this 'management' approach was the view that remediation of the non-point pollution inputs to the bathing zone, which frequently dominated the total FIO flux at the crucial high flow periods when non-compliance was most evident, could not be achieved quickly and without significant change to current farming practice (Kay *et al.* 2007). However, the pollution loading from the farming sector could not be ignored in view of its likely loading of zoonotic pathogens derived from livestock (such as *Giardia* spp., *Cryptosporidium* spp. and *E. coli* O157). Thus, public health protection was best achieved through real-time warnings to inform the public of this risk of predominantly non-human FIO sources. It was considered acceptable for the numerical compliance assessment not to use any sample results acquired during the period for which an advisory was in force in the calculation of the 95th percentile value. Thus, the risk assessment implied by the calculation of the 95th percentile value would only relate to the period during which the public were exposed to the recreational water with the approval of the regulatory authorities.

This is perhaps the most radical aspect of the new guidelines, specifically, the departure from the 'traditional' regulatory approach which requires strict numerical limits derived from agreed sampling and analytical protocols. It does, however, provide a means of establishing health evidence-based numerical guidelines at the times of bather exposure, even in an area where environmental background variability would cause a beach to fail because of adverse water quality during periods when bather exposure was likely to be low due to the very climatic conditions causing the water quality deterioration.

15.2 CURRENT DEVELOPMENTS

The Commission of the European Communities (CEC) have been the first agency to incorporate elements of the numerical values and the 'management' approach into a revised EU Directive on bathing waters (Wiedenmann 2003; CEC 2002, 2004, 2005, 2006). The Commission also utilized research findings from German epidemiological work (Wiedenmann *et al.* 2006) initiated in response to the observation by the WHO expert advisers that the epidemiological base for the 2003 WHO GSRWE was uncomfortably narrow (because it was based on north European marine waters; Fleisher *et al.* 1996; Kay *et al.* 2004). The German epidemiological studies were conducted using, as far

as possible, an identical protocol and questionnaire survey to the earlier United Kingdom randomised trials to ensure data compatibility as recommended by WHO.

The process of European Union (EU) Directive revision has generated intense scrutiny by the policy and scientific communities which has focused on both the CEC proposals and the scientific basis of the WHO Guidelines themselves (Kay *et al.* 2001). Interestingly, the United Kingdom Government's regulatory impact assessment for the proposed Directive suggested that the financial impact on the United Kingdom, as an EU Member State, would be almost neutral if a management system could be established to facilitate discounting of up to three samples per year during adverse weather conditions (DEFRA 2003a).

In the expanding EU, implementation of the Water Framework Directive (WFD), together with proposed changes to the systems of financial support for the farming community, offer potential means of reducing diffuse pollution from agriculture and its impact on both recreational and shellfish harvesting waters (Anon 2000, 2003a,b; Kay *et al.* 2007). Under Article 11 of the Directive, Member States are required to design a 'programme of measures' to achieve the standards defined in the daughter Directives listed in Annex VI which includes both the Bathing Water and the Shellfish Hygiene Directives. Thus, for the first time, the mandatory requirement for the integrated control of faecal indicator fluxes from both diffuse and point sources has been formally enshrined into environmental legislation in Europe.

This has significant implications for farming communities in Europe. However, there are policy developments which could offer significant opportunity for amended farming practices in key catchments draining to recreational and shellfish harvesting waters. The policy change is the revision of the Common Agricultural Policy and the principal drivers are the decoupling of farm support payments from production or 'headage' and the introduction of 'cross compliance' (a new explicit linkage between the farmer's 'performance', in animal welfare and environmental management, and the receipt of the 'single farm payment'). Whilst not currently linked to potential effects on 'protected areas' as defined in Annex VI of the EU WFD, it is difficult to envisage that this established mechanism would not be utilized if an EU Member State was at risk of infraction proceedings by the CEC for non-compliance with statutory criteria in Directives covering bathing and shellfish harvesting waters.

In the United States, the Clean Water Act (CWA) enshrines many of the same principles as the WFD (Horn *et al.* 2004) but its implementation precedes the EU legislation by over a decade, thus, providing some interesting insights. Where water quality is defined as *impaired* under the CWA and fails to reach target levels, the CWA requires that a Total Maximum Daily Load (TMDL)

assessment is undertaken to rectify the impairment (parallel to the WFD Article 11 '*programme of measures*'). Some 64 628 water quality '*impairments*' were reported between January 1996 and June 25 2007 and 25 255 TMDLs were approved by the United States Environmental Protection Agency (USEPA) over the same period (Elshorbagy *et al.* 2005). The top five reasons for water quality impairment leading to an agreed TMDL have been: 'microbial pollutants and pathogens' (in fact, FIOs impacting on bathing and shellfish harvesting waters) (5111 TMDLs); heavy metal pollution (5072 TMDLs); nutrients (3521 TMDLs); sediments and siltation (2682 TMDLs); and organic enrichment and low dissolved oxygen (1425 TMDLs). Some 4525 TMDLs, for all impairment causes, were approved by USEPA in the single fiscal year to 30 September 2006 (Hyer and Moyer 2004; Kay *et al.* 2006).

It is interesting to note that microbial water quality '*impairments*' of bathing and shellfish harvesting waters were the most common reasons for US TMDL studies, suggesting a higher US prominence for this area than, for example, nutrients, pesticides and oxygen demand which have all received far more attention to date by the EU regulators and policy makers addressing the implementation of the WFD (DEFRA 2002, 2003b).

Kay *et al.* (2006) reviewed the operation of microbial TMDLs in California, USA and concluded that the longer US regulatory experience with examination of catchment microbial dynamics through TMDL assessments had not, to date, produced more operationally useful empirical science or modelling approaches which could be applied in the United Kingdom. In effect, many US authorities were defining FIO '*discharge*' concentration limits for discharges to streams and coastal waters (in TMDL terminology the '*concentration-based pollutant allocations*') which were simply set at the allowed environmental '*receiving water*' concentrations required for recreational and shellfish harvesting waters for relevant discharges (a geometric mean faecal coliform concentration in agricultural and surface drainage discharges to tributary streams of <200 100 ml^{-1} and a 90th percentile for faecal coliforms in direct discharge to the coastal water of <43 100 ml^{-1}). Waste water treatment plants and boats were required to achieve a faecal coliform median of zero 100 ml^{-1}. The TMDL study examples described by Kay *et al.* (2006) did not address the spatial and temporal characteristics of the inputs or their fluxes which are more relevant than 'concentration'. Nor was the feasibility of achieving these criteria addressed. Additionally, sampling programmes were recommended which could not capture data on the hydrological events which studies world-wide have suggested account for $>90\%$ of the catchment-derived faecal indicator flux from diffuse source pollution (Kay *et al.* 1999; Lee *et al.* 2002; Lee and Kay 2006).

15.2.1 Real-time prediction of water quality

15.2.1.1 Simple univariate 'trigger' systems

The United Kingdom regulatory community has been quick both to assess the impacts of the WHO 'management' approach to bathing water regulation and to field-test systems designed to communicate the results of real-time prediction. In Scotland, a new system of 'signage' has been tested which relays the predicted water quality on each day during the bathing season to the beach front or adjacent car park (McPhail and Stidson 2004). This simple system is based on river flows and rainfall in upstream catchment areas and the Scottish Environmental Protection Agency (SEPA) simply use whichever trigger parameter best predicts non-compliance at the identified bathing beach. Data from telemetric rainfall and stream flow gauges in the contributing catchment are received in a central control room where scientific staff decide if the information received would be expected to produce non-compliance based on historical data (Plate 15.1). After initial misgivings within local communities dependent on visitors using bathing waters, subsequent reaction to this signage system has been generally positive.

Plate 15.1 Real time prediction signs as installed at bathing water locations in Scotland, United Kingdom.

15.2.1.2 Multivariate regression based systems

Multivariate regression to underpin prediction was first used in the United Kingdom at the Fylde beaches in the North West of England where the intention was to explore the reasons for continued non-compliance following significant infrastructure expenditures (Crowther *et al.* 2001). The multivariate approach has, however, progressed in the United Kingdom with studies in the Cardiff Bay impoundment where a simple operational spreadsheet model has been employed to predict real-time water quality from calibration data acquired in the previous year. Figure 15.1 shows set of predicted and observed data for 2004 (Stapleton and Kay 2004). Artificial neural network modelling has also been employed with good prediction within the calibration range but unproven inter-year transferability (Brion *et al.*, 2005; Kashefipour *et al.* (2005). Others have employed a hydrodynamic modelling framework to underpin associated health risk assessment (Elliott 1998; Harris *et al.* 2004).
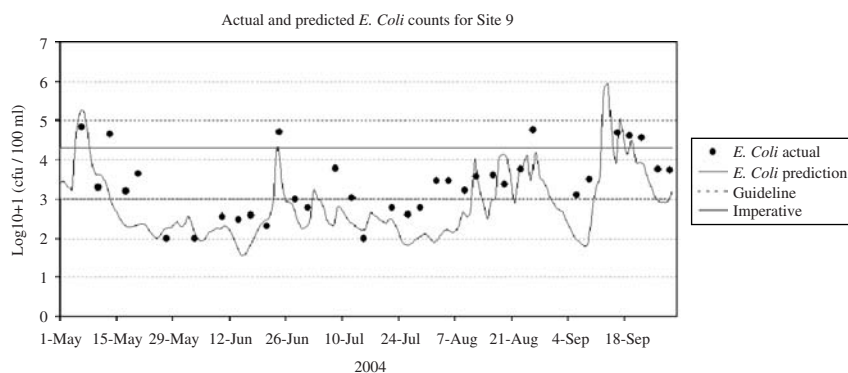
Figure 15.1 Actual and predicted concentrations of *E. coli* in Cardiff Bay, United Kingdom; plotted against EU recreational water criteria (Directive 160/76/EEC).

In North America, the US Geological Survey have issued guidance on real-time prediction options for the beach management communities (USGS 2003, 2006a, 2006b) which recommends a multivariate approach to prediction. A similar conclusion was reached following a three-year United Kingdom research programme funded by the EU (Anon 2006). Perhaps the principal limitation in any modelling study where the outcome variable used to calibrate the modelling system is a microbial determination in water or shellfish flesh. The principal reason for this is the imprecision in microbial enumeration which increases the random stochastic disturbance term and, thus, reduces the

explained variance. There is, thus, considerable research potential in enhancing the precision of this dependent variable by, for example, replicate enumerations where new data sets are being specifically generated to underpin predictive modelling.

15.2.2 Source apportionment studies

Most modelling attention to date has focused on the nearshore zone (Jin *et al.* 2003) with very little research and monitoring effort directed to define the complex and highly episodic mix of inputs from both point and diffuse terrestrial sources (Ferguson *et al.* 1996; Fraser *et al.* 1998; Ferguson *et al.* 2003a; Ferguson *et al.* 2003b; Ferguson *et al.* 2005; Ferguson 2005; Jamieson *et al.* 2003; Jamieson *et al.* 2004a; Jamieson *et al.* 2004b; Jamieson *et al.* 2005a; Jamieson *et al.* 2005b).

Early catchment-scale investigations in this area were initiated to explain continued compliance problems (impairments) following very significant expenditures on sewage treatment. Perhaps simplistically, it had been assumed that emerging effluent treatment technologies such as ultraviolet (UV) disinfection would effectively 'cure' impairment problems by removing the bulk of culturable faecal indicators from the effluent stream. When the first UV treatment system installed in Europe failed to guarantee bathing water compliance with Directive 76/160/EU criteria for coliform organisms, the search for non-outfall sources of faecal indicators and the associated 'integrated catchment studies' commenced (Wyer *et al.* 1994; Wyer *et al.* 1996; Wyer *et al.* 1997; Wyer *et al.* 1998; Wyer *et al.* 1999).

These investigations have produced a series of key observations and findings which can be summarized as follows:

- non-compliance or impairment is most often associated with rainfall events and the associated transport of faecal indicators into the nearshore zone;
- such short-term 'events' occupy a small proportion of the harvesting (and/or bathing) 'season(s)' but the flux of FIO pollution causing impairment will be delivered in these discrete periods;
- historical archive data describing this condition (the crucial rainfall induced fluxes of pollutants) is often absent, more importantly, historical 'compliance' data, collected according to a regular sampling programme will systematically under-represent this condition and, as a consequence, the management utility of such historical archive data is often limited, if not potentially misleading;

- most catchment systems have a mix of human (sewage) and animal (agriculture and wildlife) sources of faecal indicator organisms;
- under dry weather conditions, streams transporting diffuse pollution fluxes from livestock in catchment systems exhibit very low FIO concentrations, (faecal coliform concentrations of 104 to 103 per 100 ml);
- even very small and apparently pristine stream waters draining livestock areas, with little or no human sewage inputs, can exhibit FIO concentrations in higher flow conditions similar to those observed in a dilute sewage spilling from a combined sewage overflow (coliform concentrations of 105 to 106 per 100 ml);
- treated sewage effluent will exhibit FIO concentrations determined by the treatment systems and the flow through the treatment plant, but, during low flow conditions, the treated sewage effluents are often the dominant source;
- treated sewage effluent may exhibit very different FIO concentrations following rainfall events, both concentration reductions (due to dilution) and increases (due to increased plant loadings) have been reported (Wyer *et al.* 1998) and generalizations in this area are inappropriate due to the site specific nature of the sewerage systems installed; and
- where the sewage system is designed to accommodate 'combined' surface drainage and foul sewage there will generally be some system of overflows from the sewerage system if it becomes full (termed 'combined sewage overflows' – CSOs), or from a holding tank used to provide buffering storage before the sewage plant (termed storm tank overflows – STOs). Under event conditions such CSOs and STOs will discharge to rivers or directly to the coast and these may represent a considerable flux of organisms which commonly enter a river or stream during the early part of the high flow event.

Management information on this complex input pattern is required to target appropriate expenditures on point (mainly human) and diffuse (mainly animal) source control strategies. The key management information required is the proportions of the flux derived from all potential inputs during both low and high flow conditions. Clearly, this requires samples to be acquired from streams and the sewerage infrastructure during event conditions which is logistically difficult and requires aseptic hand sampling if the resultant data are to be credible for operational purposes (Anon 2002).

Simple pie charts can represent this source apportionment and Figure 15.2 shows this representation for the Irvine catchment in Scotland, United Kingdom. This represents all inputs to the bathing zone comprising a crude discharge via

a long sea outfall, CSOs and STOs and diffuse agricultural sources. This catchment is predominantly used for livestock rearing but also contains the settlements of Kilmarnock and Irvine which, at the time of the study, produced a screened primary treated effluent stream from approximately 200 000 persons. Figure 15.3 shows the flux pattern for this catchment represented in hourly FIO delivery over an eight-week period in the bathing season. This pattern suggests that in dry weather conditions, the marine discharge from the sewage treatment works dominates the input but, during wet conditions after rainfall, the total flux (middle plot) increases rapidly and the diffuse component becomes a much larger contributor to the total FIO flux to the bathing zone (lower plot). Interestingly, the CSO component (principally from the Kilmarnock sewerage infrastructure) represents a relatively small component of the total flux and does not dominate even during the brief periods of CSO discharge.

These flux diagrams were produced by intensive sampling of high flow and low flow water and effluent qualities for a period of eight weeks, together with stream and effluent flow monitoring to calculate the hourly flux information. With this base information for a site it is relatively easy to insert alternative concentrations for different sewage treatment options. Characteristic concentrations for such effluents can be derived from Kay *et al.* (2008).

The rural pattern seen in the Irvine catchment contrasts with more urban delivery from conurbations. An urban catchment flux study has been completed in the Ribble catchment area in Lancashire, United Kingdom (Wither *et al.* 2005; Stapleton *et al.* 2008). Pie charts, hourly flux plots and a bar chart representation for an eight week summer period in this catchment are shown in Figures 15.4, 15.5 and 15.6. This urbanised area also exhibits a rapid increase in the diffuse source, catchment-derived 'River Ribble' contribution following rainfall events. However, the bar chart in Figure 15.6 clearly suggests that the majority of high flow inputs to the estuary are in fact attributable to CSOs and STOs. This was certainly a counter-intuitive finding but clearly the balance of the various inputs is important management information if expenditures are to be appropriately targeted between, for example, (i) storage to limit CSO discharges weighed against (ii) disinfection of treated sewage effluents and (iii) implementation of pollution control through farm-scale 'best management practices' (BMPs).

15.2.2.1 Using satellite data for catchment delivery modelling

The approach described below has employed catchment models of faecal indicator delivery to provide hourly input sequences from riverine and infrastructure sources to act as input variables for nearshore mathematical modelling (Fraser *et al.* 1998; Kay *et al.* 2005). The catchment FIO flux models presented
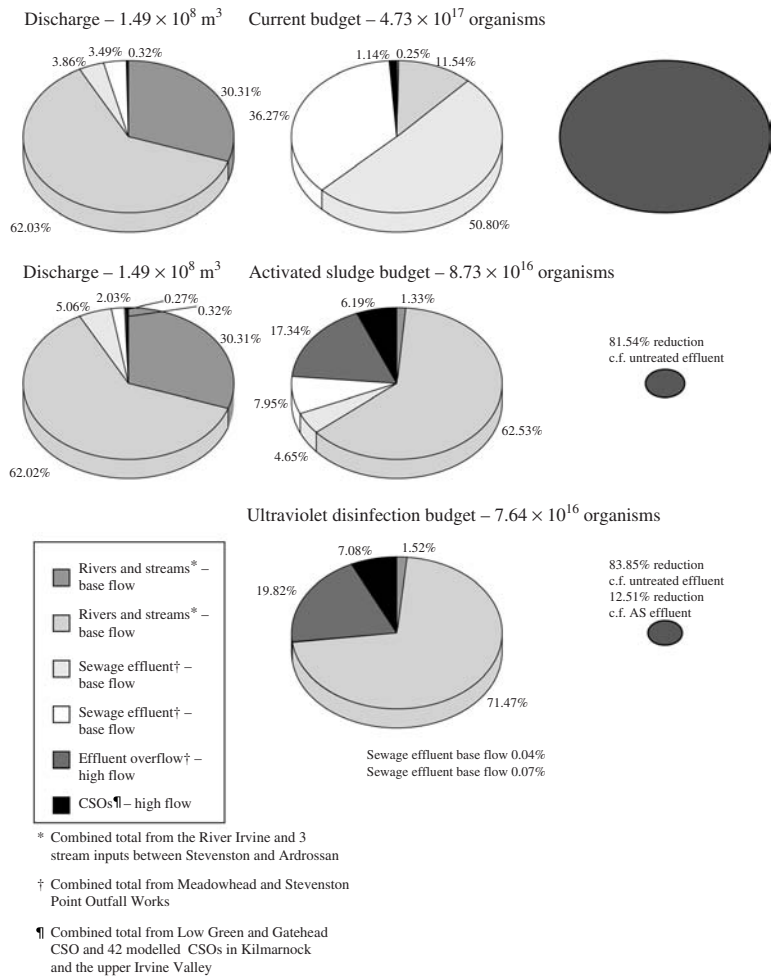
Figure 15.2 Pie charts of faecal coliform budgets discharged to coastal waters during the summer bathing season into Irvine Bay, Scotland, United Kingdom.

below are based on multiple regression equations used to predict the low and high flow faecal indicator geometric mean concentrations entering the nearshore zone. These are calibrated from empirical data describing low flow and high flow geometric mean faecal indicator organism concentrations (dependent variables) and satellite-derived land use data (predictor variables). These data
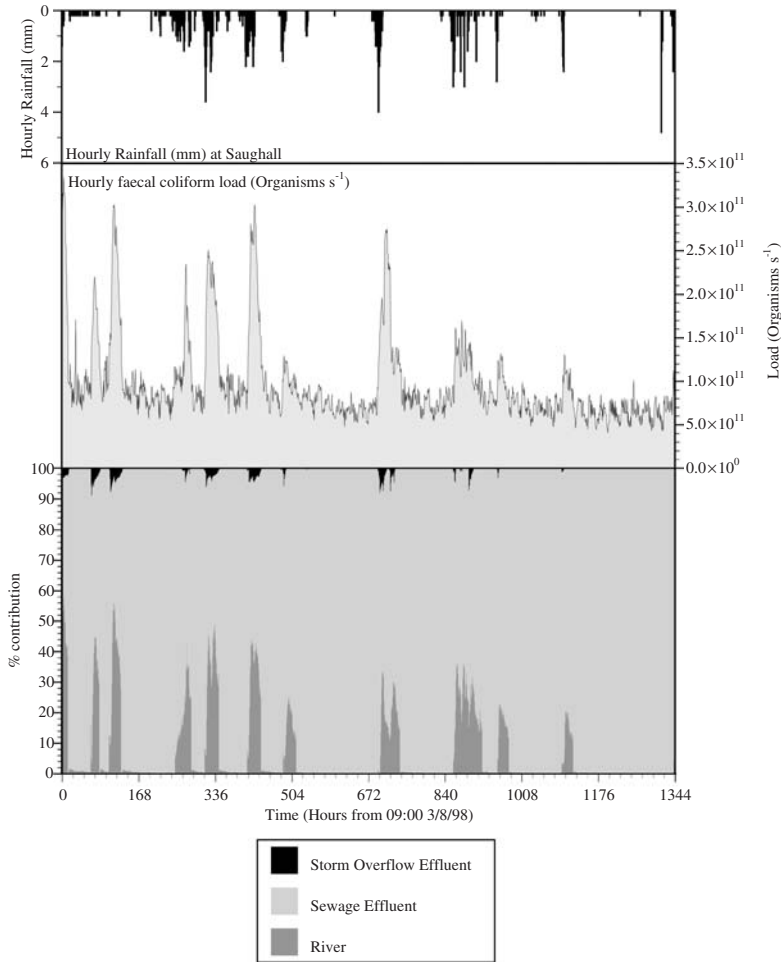
Figure 15.3 Hourly flux plot of faecal coliform for Irvine Bay, Scotland, United Kingdom.

have been used in both rural and urban catchments (Crowther *et al.* 2002; Crowther *et al.* 2003; Kay *et al.* 2005) to predict high and low flow FIO geometric mean values which, when combined with hourly discharge volumes, can provide FIO flux estimates.

In the United Kingdom studies, this process has utilized a digital terrain model (DTM), at a cell resolution of 25 m, derived from Ordnance Survey (OS)
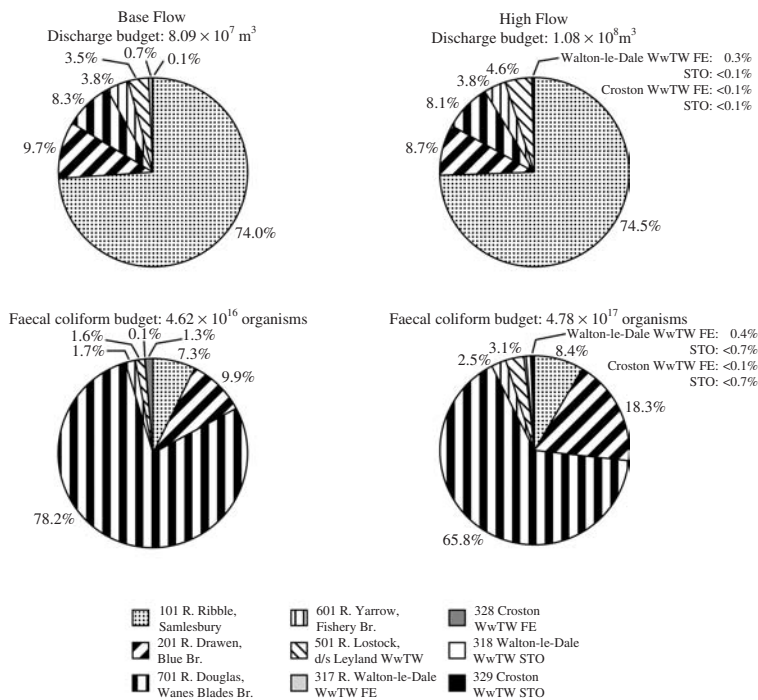
Figure 15.4 Pie charts representing the proportion of different riverine and sewage infrastructure inputs to the Ribble estuary, Lancashire, United Kingdom.

digital contour data. This is modified to create a DTM using standard ARC/INFO procedures to fill in anomalous sinks that often occur along the valley floors in the DTM. This 'filled' DTM can be used as a basis for flow path analysis and derivation of a raster drainage network. Subcatchment outlet points are positioned on this network as close as possible to the actual sampling points. Standard ARC/INFO routines are then used to derive the topographic watershed boundaries for each subcatchment.

The digital map of land cover, at 25 m resolution, used to date, is derived form of the Centre for Ecology and Hydrology (CEH) 1990 (or 2000) land cover maps. These are generated from remotely-sensed (Landsat) imagery and divide land cover into 17 classes (Table 15.1). Additional digital land cover data for the United Kingdom are available from OS 1:50 000 colour raster maps. A common 25 m resolution cover can be generated based on these maps as described below.

Accurate land cover and associated water quality data from previous detailed field mapping programmes are available for >200 United Kingdom
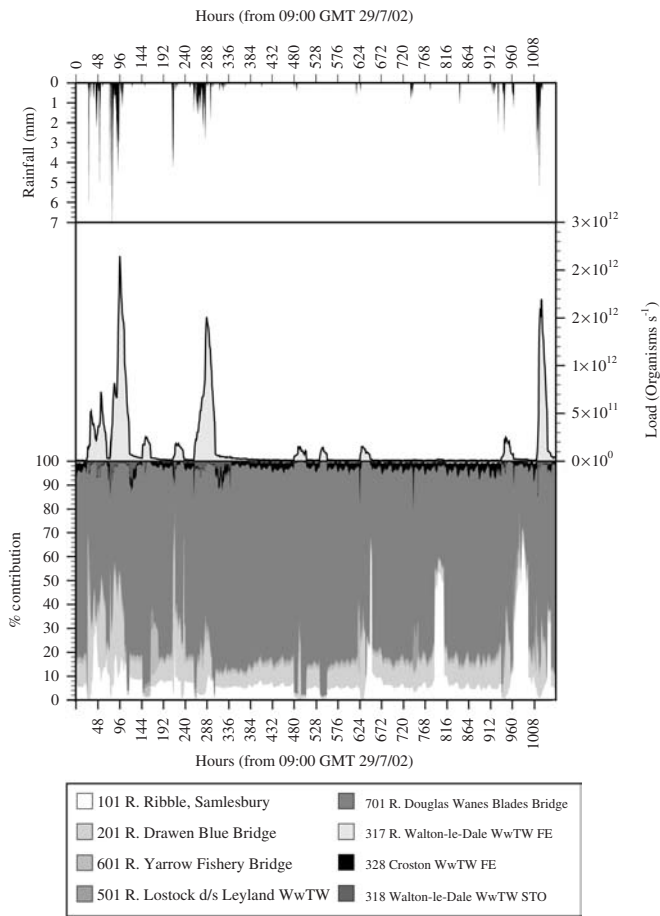
Figure 15.5 Hourly flux of faecal coliform organisms derived from different riverine and sewage treatment plants impacting on the Ribble estuary, Lancashire, United Kingdom.

subcatchments in five study areas in England and Wales. The 17 CEH land cover classes are categorized according to the seven principal land use classes attributed during field surveys (Table 15.1). It should be noted, however, that comparison of the CEH land cover data with the field survey data in the 200 subcatchments showed some significant discrepancies, particularly with regards to built-up land, woodland and improved pasture, suggesting inaccuracies in the remotely sensed data.

Faecal coliform loads discharged to each subcatchment
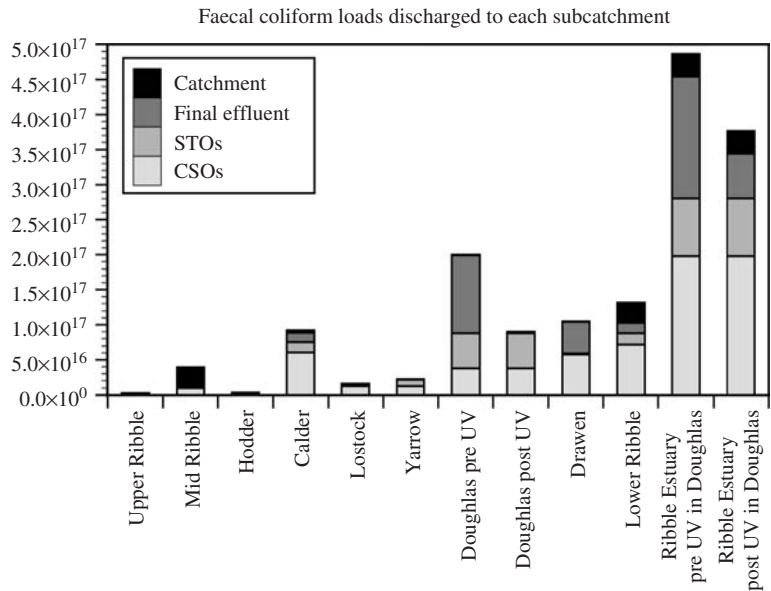
Figure 15.6 Bar chart representation of the faecal coliform loadings impacting on the Ribble estuary, Lancashire, United Kingdom.

These limitations in the satellite land cover data have been addressed as follows. First, maps of built-up land and woodland are generated based on the unique colours used to depict these land use types in the OS 1:50 000 map. The woodland area extracted from the OS 1:50 000 map data was found to correspond very closely with the field survey data from the 100 subcatchments. Three problems were identified in extracting the built-up land: (i) only buildings are identified and not roads, gardens, and similar infrastructure which would conventionally be classified as part of built-up areas; (ii) some public buildings are excluded because they are depicted using different colours; and (iii) lettering is often superimposed on built-up areas, further reducing the area of built-up land extracted. To quantify this under-estimation in one United Kingdom study area (the Ribble catchment; Kay *et al.* 2005), 25 500 × 500 m squares, which would be conventionally mapped as 100% built-up, were selected from the OS 1:50 000 raster map set for England and Wales. The area of built-up land was extracted as outlined and, on average, was under-represented by a factor of 3.11.

Comparison of the proportion of improved pasture identified in the 100 subcatchments with that derived from the CEH satellite land cover data showed

Table 15.1 Details of the Centre for Ecology and Hydrology (CEH) 1990 land cover classification (17 classes) and the corresponding land use type to which they have been attributed.

CEH class	Description	Land use type to which the CEH class has been attributed[a]
0	Unclassified	Unclassified
1	Sea, coastal waters and estuaries, inland to first bridging point or barrier	Other
2	Inland fresh waters and estuarine waters above the first bridging point or barrier	Other
3	Bare coastal mud, silt, sand shingle and rock, including coastal accretion and erosion features above high water	Other
4	Intertidal seaweed beds and salt marshes up to normal levels of high water spring tides	Other
5	Semi-natural, mostly acid, grasslands of dunes, heaths and lowland-upland margins + Montane/hill grasslands, mostly unenclosed nardus/molinia moorland	Rough grazing
6	Pastures and amenity swards, mown or grazed, to form a turf throughout the growing season + Meadows, verges, low intensity amenity grasslands and semi-natural cropped swards, not maintained as short turf	Improved pasture
7	Lowland marsh/rough grasslands, mostly uncropped and unmanaged, forming grass and herbaceous communities, of mostly perennial species, with high winter litter content + Ruderal weeds colonising natural and man-made bare ground + Felled forest, with ruderal weeds and rough grass	Rough grazing
8	Upland, dwarf shrub/grass moorland + Lowland dwarf shrub/grass heathland	Rough grazing
9	Upland evergreen dwarf shrub-dominated moorland + Lowland evergreen shrub-dominated heathland	Rough grazing
10	Bracken-dominated herbaceous communities	Rough grazing

(*continued*)

Table 15.1 *Continued*

CEH class	Description	Land use type to which the CEH class has been attributed[a]
11	Deciduous scrub and orchards + Deciduous broadleaved woodland and mixed woodlands	Other
12	Conifer and broadleaved evergreen trees	Woodland
13	Lowland herbaceous wetlands with permanent or temporary standing water + Lowland herbaceous wetlands with permanent or temporary standing water	Rough grazing
14	Arable and other seasonally or temporarily bare ground	Arable
15	Suburban and rural developed land comprising buildings and/or roads but with some cover of permanent vegetation	Built-up
16	Industrial, urban and any other developments lacking permanent vegetation	Built-up
17	Ground bare of vegetation, surfaced with 'natural' materials	Other

[a] Based on detailed notes that accompany the classification scheme.

a strong linear relationship. However, improved pasture is under-represented where a high proportion is present and over-represented where very little is present. The final map used to derive subcatchment land use needed to drive the FIO flux models requires percentage areas of improved pasture, rough grazing, arable and 'other' land use categories and this element is generated based on the CEH land cover data. However, given the uncertainties listed above, the built-up and woodland categories in the CEH data source are reclassified as 'unclassified' at this stage. This map is then amalgamated with the built-up and woodland areas extracted from the OS 1:50 000 map, with the latter map categories being given precedence (thus a cell of improved pasture on the CEH map classified as woodland on the OS 1:50 000 map would be classified as woodland in the final data set).

The resultant areas of each land use type in each subcatchment are then adjusted. First, unclassified land is re-allocated to the improved pasture, rough grazing, arable and 'other' categories in proportion to the area of these land use types identified within each subcatchment (i.e. no adjustments are made to the areas of built-up and woodland categories). Second, the area of built-up land is increased by the factor of 3.11, the area of land required for this being subtracted proportionately from the areas of improved pasture, rough grazing, arable and other categories.

In addition, land areas upstream of the outlets of all identifiable lakes and reservoirs (from the OS 1:50 000 maps) is defined. Land use within these areas is reclassified as 'reservoir catchment'. This additional classification attempts to account for low faecal indicator organism concentrations that would be associated with die-off and sedimentation processes within such water bodies (Kay and McDonald 1980) and the resultant effect of water quality at the subcatchment outlet not reflecting the land cover pattern within the subcatchment. It should be noted that, whilst these procedures produce much more accurate data on the overall proportions of different land use types within each subcatchment, some adjustments made (such as built-up land) are not location specific and cannot therefore be represented on a map.

Outputs of this approach for a recent study in the Ribble catchment, United Kingdom are shown in Figure 15.7 which also includes sewage treatment works in the study area.

15.2.3 Linked catchment and nearshore modelling

The compartmentalization of modelling into terrestrial (catchment) and nearshore (hydrodynamic) is often evident due to the different communities involved. This can, however be counter-productive because both bathing and
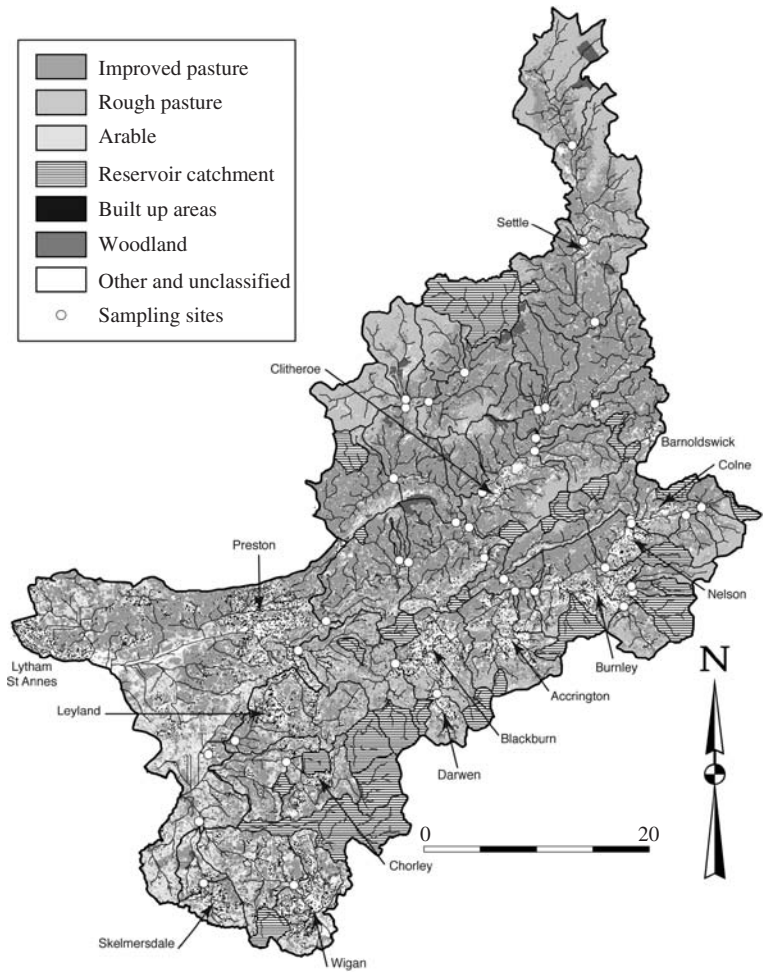
Figure 15.7 Satellite derived land use data for the Ribble catchment, Lancashire, United Kingdom. Source: Wyer *et al.* (2003).

shellfish harvesting waters are impacted by terrestrial pollutant fluxes from the land surface. Previous modelling in this area has suffered from a lack of understanding of (i) the nearshore (i.e. shallow water) hydrodynamic and microbial fate and transport processes and (ii) the highly dynamic FIO fluxes derived from terrestrial point and diffuse sources. It could be argued that linked catchment and near shore models are required to facilitate accurate prediction of

FIO concentrations at relevant compliance locations and to drive appropriate remediation strategies which will increasingly have to address issues of altered farming practice and their likely impacts on compliance (Kashefipour *et al.* 2006; Kay *et al.* 2007).

Linked catchment and nearshore modelling has been undertaken in three United Kingdom study sites with the aim of predicting recreational water quality. These are: the Ayrshire coast (Wyer *et al.* 2001; Kashefipour *et al.* 2006), the Severn estuary (Stapleton *et al.* 2004; Wyer *et al.* 2007; Yang *et al.* 2007) and Carmarthen Bay (Wyer *et al.* 2004).

These investigations have clarified the interaction between event driven water quality changes on microbial dynamics in riverine and nearshore waters, particularly the role of turbidity derived from entrained riverine sediments on faecal indicator survival, and the potential for real-time decay functions in hydrodynamic nearshore models for prediction of faecal indicators in the compliance zone for bathing and shellfish harvesting waters (Sinton *et al.* 2002; Kay *et al.* 2005; Wilkinson *et al.* 2006).

There has, to date been less attention to waters often favoured for shellfish cultivation such as sea lochs and inlets where tidal water exchange may be less pronounced and the hydrodynamic modelling challenges are significant.

15.2.4 Remediation of faecal indicator fluxes from coastal catchments

There have been very few long-term empirical studies which have sought to quantify the remediation potential of the principal policy levers available to reduce microbial pollutant fluxes (Shreeram and Mostaghimi 2002). Undertaking such assessments is complicated by: (i) seasonality in faecal indicator flux, with temperate livestock rearing areas exhibiting a summer peak in high flow stream water concentrations (Rodgers *et al.* 2003); (ii) poor information on the likely time taken for measures designed to reduce microbial flux to become effective at the catchment scale.

In one of the rare longitudinal (before and after) catchment-scale studies designed to quantify the effect of a BMP (in this case cattle exclusion from catchment streams) on FIO flux from a 56.7 ha drainage basin, Line (2003) reported data derived from a 7.5 years sampling period which suggested 65.9% and 57.0% reductions in faecal coliform and enterococci export respectively. They also reported that the provision of an alternate water supply without fencing was not effective in producing FIO reduction (see also Shreeram and Mostaghimi 2002). In a two-year United Kingdom longitudinal investigation of

FIO export through a period of de-stocking due to an outbreak of foot and mouth disease, Chalmers*et al.* (2005) and Sanders *et al.* (2005) reported a surprisingly slow improvement in water quality following the most drastic BMP of >95% stock removal from the 254.6 ha Caldew catchment in Cumbria, United Kingdom. A longitudinal study at Brighouse Bay in Scotland, United Kingdom, examined the effects of BMPs on water quality in catchment streams and at an adjacent bathing water beach. The principal BMP was stream bank fencing to create a riparian buffer strip (RBS) with associated provision of drinking troughs. Farm dirty water containment was also implemented. The stream water quality data suggested extreme seasonality with the summer period having markedly higher FIO concentrations in catchment streams. However, comparison with an unmodified adjacent control catchment suggested a 66% reduction in *E. coli* summer high flow export coefficient (in $cfu.m^{-2}.hr^{-1}$) with a parallel 81% reduction in intestinal enterococci export. Detailed monitoring through a rainfall event in the post-remediation period suggested that even this improvement would be insufficient to guarantee bathing water compliance with Directive 160/76/EEC (Dickson *et al.* 2005). The separate effects of RBS and steading dirty water control have been addressed in a longitudinal study of 60 monitored catchments in Scotland by Kay *et al.* (2005). Here, significant improvements were recorded in FIO flux when compared to 'control' catchments but a relatively high intensity of 'measures' was required (>30% of stream bank length protected by RBSs).

Bacterial source tracking has been employed by Hyer and Moyer (2004) to inform TMDL studies in the USA and Pond *et al.* (2004) provide an excellent overview of the potential for the source tracking methods currently available to contribute to FIO flux source apportionment. These methods use either (i) species and or sub-species of organisms thought to be associated with faecal matter from humans or defined animal groups or (ii) chemical markers indicative of human sewage. There is currently no single and definitive approach with which to identify exact proportions of human and animal derived FIOs, but this area is developing rapidly and may provide operationally useful data in the medium term. However, parallel testing of source tracking, where traditional source flux apportionment data are available, suggests that the essentially qualitative tracking information does not provide additional explanatory power (Stapleton *et al.* 2007).

15.3 CONCLUSIONS

A series of related policy and public health agendas are emerging in the general area of 'catchment microbial dynamics' (Kay *et al.* 2007) which is a relatively

immature field when compared to other water quality modelling areas such as nutrient flux assessment. This is of direct relevance to both shellfish and bathing waters. Effort, at the EU scale, is evident to develop integrated modelling strategies able to address the needs of WFD implementation (Moore and Tindall 2005) and scientists in the USA are similarly engaged through modelling platforms such as BASINS (Tong and Chen 2002). However, operationally useful, fully white box, deterministic and process based faecal indicator models able to predict the effects of individual remedial 'programmes of measures' or BMPs on catchment scale FIO fluxes simply do not exist at the present time.

Science activity is developing to address this emerging agenda but some fundamental questions remain such as:

i.   How long do the FIOs live in river water under different flow and turbidity conditions? This is a vital question if this highly non-conservative parameter is to be modelled at a catchment scale. Recent developments in nearshore waters have employed real-time decay rates predicted by light intensity and turbidity and a similar approach for riverine matrices is needed (Kay *et al.* 2005).

ii. What are appropriate FIO export coefficients for different land use types to use in diffuse source models, how do such coefficients vary with season in different farming areas (Kay *et al.* 2008)?

iii. What are the likely reductions in FIO flux achievable through implementation of feasible land management interventions (BMPs) and will such interventions produce compliance of shellfish harvesting areas and bathing waters with existing and future standards?

iv. How do we balance the different interventions available to reduce faecal indicator fluxes from the sewerage network, principally disinfection of treated effluents against additional storage to reduce spills from CSOs and STOs; such deliberations often depend on sewer modelling studies but the poor precision of volumetric estimates from such models is often insufficiently based on transparent empirical data to facilitate robust assessment of model reliability.

v.   To what extent are currently available commercial nearshore hydrodynamic models capable of real-time prediction? This question is being raised as the traditional engineering consultancies which have familiarity with hydrodynamic water quality modelling address the emerging agenda of real-time water quality management. The modelling tools available may, however, lack precision in shallow water environments and, more importantly, peer reviewed data to underpin assessment of key evaluation criteria such as the model explained variance or R^2 term.

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16

Microbial modelling in coastal environments and early warning systems: useful tools to limit shellfish microbial contamination

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To reduce human health risks and significant revenue losses due to the closure of shellfish-farming areas, it is necessary to maintain good water quality and food safety in marine environments. The major pathways introducing enteric bacteria and/or viruses into coastal areas are via urban and agricultural effluents. These microorganisms, and others naturally residing in marine waters such as

Vibrio spp., may subsequently contaminate filter-feeding shellfish (Lee and Younger 2002; see Potasman *et al*. 2002 and Butt *et al*. 2004 for reviews on potential pathogenic microorganisms in shellfish). Shellfish are often consumed raw or undercooked and thus have been implicated in some foodborne diseases (Lipp and Rose 1997; Feldhusen *et al*. 2000; Yam *et al*. 2000). By implementing shellfish farming regulations, the incidence of enteric shellfish-borne diseases, especially *Salmonella* spp. outbreaks, has been considerably reduced worldwide (Richards 2003). However, enteric viruses are still detected in shellfish (Le Guyader *et al*. 1998; 2000) and have been implicated in shellfish-borne outbreaks after oyster consumption, in some cases with the shellfish meeting European Union (EU) bacteriological standards (Gill *et al*. 1983; Richards 1985; Dowell *et al*. 1995; Otsu 1999; Le Guyader *et al*. 2006). This suggests that, if conventional depuration can remove faecal indicators and bacterial pathogens such as *Escherichia coli* or *Salmonella* spp. (Marino *et al*. 2003), it is less effective for eliminating faecal viruses from shellfish (Lee and Younger 2002; Loisy *et al*. 2005a). Thus, growing shellfish in an environment free from microbial contamination seems to be a more suitable solution than depurating potentially contaminated filter feeders. The use of tools such as statistical or hydrodynamic models applied to faecal microorganisms and early warning systems for shellfish production sites, could predict microbial contamination in shellfish-harvesting areas (Grange 1999; Tattersall *et al*. 2003; Martinez-Urtaza *et al*. 2004; Pommepuy *et al*. 2006).

16.1 MODELLING ENTERIC MICROORGANISMS IN COASTAL ENVIRONMENTS

When entering a coastal environment, faecal microorganisms, free and bound, undergo different processes: (i) physical dilution induced by currents and mixing, (ii) physico-chemical conditions: sunlight irradiation, salinity, temperature, pH and nutrient availability, which can result in bacterial stress or viral degradation (Trousselier *et al*. 1998; Rozen and Belkin 2001), and (iii) biotic effects, including competition with other microorganisms and grazing (Barcina *et al*. 1991; Rozen and Belkin 2001).

In order to evaluate and/or to predict the impact of such microbial contamination in bathing or shellfish-harvesting areas, modelling tools have been developed. They are mainly dedicated to faecal microbial indicators such as faecal coliforms (FC) (Roberts and Williams 1992; Ribeiro and Araujo 2002), *E. coli* (Tattersall *et al*. 2003; Armisen *et al*. 2006), enterococci (Bell *et al*. 1992) or F-RNA-specific bacteriophages and astrovirus (Riou *et al*. 2007).

The models are classified in two main categories: statistical or process-based dynamic models.

16.1.1 Statistical models

The main objectives of this type of model are: (i) to describe the microbial contamination that results from sewage inputs or non-point sources in a specific site, according to relevant environmental conditions, and (ii) to predict water quality. These models are based on linear or logistic regression analyses that link environmental parameters such as rainfall, wind or sunlight to faecal contamination, mostly to faecal coliforms or *E. coli* (EPA 1999; Crowther *et al*. 2001; Martinez-Urtaza *et al*. 2004). Their development requires large monitoring data sets of both (i) rainfall in the upstream watershed (one or more rainfall stations; rainfall characteristics: amount, duration, lag time) or other relevant environmental parameters, and (ii) faecal microorganisms occurrence at the representative monitoring station (bathing or shellfish-farming area). Then, predictive tools are built and the results obtained by rainfall based alert curve models (such as the quantity of rain during the past 24 hours, EPA 1999) or by real-time monitoring of different hydrometeorological variables (Olyphant 2005) that define the conditions of closure of shellfish-farming or bathing areas (Wither *et al*. 2005). These models could also provide cost-effective management tools for the exploratory investigation of any monitoring point that is failing to meet water quality standards. Their application to the data collected on the Fylde coast (United Kingdom) have shown significant positive and negative relations between microbial concentrations and rainfall, the tide height at the time of sampling, onshore winds and sunlight, respectively (Crowther *et al*. 2001). Furthermore, general linear modelling was performed to relate *E. coli* shellfish contamination to environmental factors in shellfish-farming areas in the United Kingdom (Lee and Morgan 2003). Regression analysis models were also used for other bacteria, including *Salmonella* spp. (Martinez-Urtaza *et al*. 2004) and *Aeromonas* spp. (Maalej *et al*. 2003) or viruses – norovirus, hepatitis A virus, human adenovirus and enterovirus (Hernroth *et al*. 2002; Formiga-Cruz *et al*. 2003) in coastal waters or shellfish.

Another modelling approach is the use of artificial neural network (ANN) models. Among the different ANN architectures, the multi-layer perceptron architecture is commonly used for prediction (Gamal El-Din and Smith 2002). According to Brion *et al.* (2005), ANN models predict the presence and absence of norovirus and other viruses in shellfish with better precision than logistic regression models. Artificial neural networks were coupled with hydrodynamic models in order to model the faecal coliform contamination of an estuarine

system (Scarlatos 2001; Lin *et al*. 2008). They assisted in identifying correlations between FC and various parameters involved such as pH, temperature and turbidity (Scarlatos 2001) and in providing rapid predictions of the FC concentrations in coastal waters (Lin *et al*. 2008). Although a regression tree (Gray and McDonell 1997) has already been used in wildlife modelling (such as distribution of *Frankia* spp. strains and symbiotic bacteria, Oakley *et al*. 2004 or Marbled Murrelets, a seabird in coastal British Columbia, Canada, Yen *et al*. 2004), this tool has not often been used to predict microbial contamination in coastal environment. However, an extension of tree regression, named "Random forests", was used to study relationships of the concentrations of *E. coli* and enterococci in bathing waters to numerous potential explanatory variables including weather, hydrological conditions and contaminant sources (Parkhurst *et al*. 2005).

16.1.2 Process-based dynamic models

Considering the limits of statistical models (including no distinction between inputs, no consideration of advection, transport, or microbial decay rate and no provision of spatial and temporal distribution), other models describing the processes were developed. For the development of a shellfish and/or water quality model, different sub models are needed: (i) a hydrodynamic model which provides knowledge of current and mixing coefficients, (ii) a dispersion model which integrates transport and diffusion of bacteria/viruses, and (iii) a biological model (microbial decay) which describes the decay of bacteria/viruses depending on environmental conditions (light, temperature and sediment).

16.1.2.1. Hydrodynamic models

In the past, the description of hydrodynamic conditions was based on physical modelling (such as tank-based simulations). Problems of scaling and cost lead engineers to use numerical models which are cheaper and potentially more efficient for water quality applications. Numerical models are useful tools to assess different discharge strategies or best location of outfall, effects of load reduction or impact of local construction.

A range of models with differing levels of sophistication were developed and adapted for a wide variety of hydrodynamics. The choice of a model depends on local hydrological features, which is a function of local dynamic processes. These models are based either on open sources (Falconer and Lin 1997; Fiandrino *et al*. 2003; Pommepuy *et al*. 2004) or on commercial packages (Roberts 1999; Kashefipour *et al*. 2002; Servais *et al*. 2007) which include user-friendly interfaces.

Usually, a distinction is made between near and far field (Monteiro *et al*. 1992; Roberts and William 1992). In the former, the mixing is due to the turbulence induced by the discharge itself (near the diffuser) and leads to a local dilution factor of 100-fold or more. Beyond the near field, the far field is the area where dispersion is due to coastal currents and mixing under the influence of physical processes such as tides, wind-induced circulation, waves or, in addition, it can be density driven. Near field models are usually termed pseudo-empirical models, based on extensive experimental studies often on multiport diffusers (Monteiro *et al*. 1992; Roberts 1999). The linkage of near field and far field solutions is particularly important in modelling deep discharges (Monteiro *et al*. 1992).

Hydrodynamic models aim to resolve fluid dynamical equations (known as Navier Stockes equations) under various levels of simplification. When the dispersion away from the outfall is mainly longitudinal, as in a well mixed estuary (Yang *et al*. 2002) or in an elongated lagoon (Steets and Holden 2003), a one dimensional model is suitable. These models are based on vertical and lateral integration of the complete set of equations. When a spatial description is needed, for example in open coastal ocean, 2D models are used if the water property can be considered as well mixed. This includes generally shallow water areas where tidal or wind induced currents generate high turbulence and vertical homogeneity (Pommepuy and Salomon 1991; Kashefipour *et al*. 2002; Pommepuy *et al*. 2004). In case of thermal and/or haline vertical stratification, complete 3D models are needed. This is usually the case of estuaries where high river discharges occur and when the surrounding area is under fresh water influence (Falconer and Lin 1997) or in calm areas where temporary stratification can occur, for example, in a lagoon (Friandrino *et al*. 2003).

Numerical models, however, have to be used carefully because they have uncertainties linked to the model itself and to the site specific application. Models have to include high level turbulence closure in complex hydrodynamic environment, realistic open boundary conditions and meteorological forcing. *In situ* measurements are generally needed to validate hydrodynamic model simulations. Simulation of currents and mixing in shellfish-farming areas to estimate contamination requires a knowledge of bathymetry adapted to the high spatial resolution needed for local application (typical mesh size in the order of 100 m) and this constraint usually requires new empirical bathymetric data acquisition (Kashefipour *et al*. 2002).

16.1.2.2 Dispersion models

Microbial dispersion is due to transport (or advection) by currents and diffusion by turbulence. Two approaches are used for dispersion models, the Eulerian

and/or Lagrangian approaches (Monteiro *et al*. 1992; Tattersall *et al*. 2003). The first one consists of the calculation of a concentration at nodes of a fixed grid by solving the continuity equation. These models need to incorporate a high level advection scheme to avoid numerical diffusion (Monteiro *et al*. 1992). The Lagrangian approach involves tracking virtual particles which are hypothesised to carry a part of the discharge. Advection of each particle is calculated by temporal integration of the local current estimated by interpolation of current components calculated according to a fixed grid. The diffusion process is simulated at a step in which each particle is submitted to a random displacement determined by the diffusion coefficient (Roberts 1999b). This approach was used, for example, to simulate particle movements from any location of the model during a mean tide within the French coastal areas in Normandy (Riou *et al*. 2007). The Lagrangian approach is potentially very efficient but becomes unfeasible for long-term modelling due to the considerable amount of particles to track. Hybrid approaches have been proposed (Monteiro *et al*. 1992) and they usually promote the use of the Lagrangian model for near field and of the Eulerian model for far field or for low contamination and spatial gradient applications.

16.1.2.3. Parameters specific to a microbial application

Microbial applications aim at describing the fate of bacteria and/or viruses in coastal waters and their concentration in shellfish due to local or diffuse microbial input. In addition to the physical dispersion model described previously, they gather a decay model in seawater and a shellfish concentration model and eventually a transport of bound organisms model.

For an application of hydrodynamic models to microorganisms, the faecal input location and their fluxes have to be specified (Pommepuy *et al*. 2006). The main faecal sources are: (i) domestic fluxes originating from a sewage treatment plant (for example, 10^{12}–10^{13} FC/s from raw sewage discharge from San Francisco, USA population 1 million inhabitants, Robert and Williams 1992; and 1.6×10^8 FC/s from a town of 30 000 inhabitants; Salomon and Pommepuy 1990), (ii) non point source from rivers (river fluxes from 9×10^6 to 5×10^{10} FC/s; Baudart *et al*. 2000) or from runoff from pastured land (Crowther *et al*. 2002; 2003; Kay *et al*. 2007), and (iii) boats (Sobsey *et al*. 2003). Currently, many studies are published for FC, but very few data are available for viruses and/or pathogenic bacteria. However, with the recent development of molecular techniques like real-time polymerase chain reaction (PCR) or Reverse Transcription-PCR (RT-PCR), quantitative inputs have been obtained for non culturable viruses or viable but non culturable pathogenic bacteria (Schoeverer

et al. 2001; Gilbride *et al.* 2006). Using real-time RT-PCR, a mean astrovirus concentration of 1×10^4 astrovirus genomes for 100 ml of raw sewage had been recorded at the outlet of a biological sewage treatment plant. This corresponded to an average flux of 3×10^7 astrovirus genomes/s (for a city of 120 000 inhabitants; Le Cann *et al.* 2003).

Microbial decay in the coastal environment

As faecal microorganisms are non-conservative elements, their fate in the coastal environment is dependent on their decay rate in that environment. Their fate depends on the bacterium itself (species, strain and physiological status), on physical and chemical characteristics encountered in the environment (temperature, salinity, organic matter content, oxygenation, pH), on atmospheric conditions (mainly sunlight irradiation) and on biotic factors (predation and competition; Chamberlin and Mitchell 1978; Martin *et al.* 1998; Troussellier *et al.* 1998; Rozen and Belkin 2001). Environmental conditions before exposure to seawater are also important, and pre-adaptation to some of the marine deleterious effects were shown to be beneficial (Dupray and Derrien 1995; Munro *et al.* 1995; Gourmelon *et al.* 1997). As for viruses, which generally act as inert particles, their survival is longer than that of bacteria and depends mainly on environmental parameters such as temperature, salinity, predation and sunlight irradiation (Bosch 1995; Wait and Sobsey 2001).

   Numerous experiments have been conducted in order to determine microbial (especially faecal bacterial indicators) decay rates in coastal environments. This decay rate is often expressed as T_{90}: the time for bacterial or viral concentration to decrease by one log unit. The major factor affecting bacterial decline in the sea was shown to be sunlight irradiation (Sinton *et al.* 1994; Rozen and Belkin 2001). Diurnal variations were recorded (Bellair *et al.* 1977; Chamberlin and Mitchel 1978). The role of turbidity on light penetration was also observed and thus its impact on T_{90} was demonstrated (Pommepuy *et al.* 1992; Alkan *et al.* 1995; Kay *et al.* 2005). A relationship between T_{90} and daily light intensity, suspended solid concentration and depth has been obtained from experimental data for *E. coli* (Guillaud *et al.* 1997). Nevertheless, results obtained from *in vitro* or *in situ* experiments are generally variable and difficult to compare because of the great variability existing among the protocols that are used. So, for a study in a defined area, it is advisable to measure microbial survival and environmental parameters such as sunlight *in situ* rather than to rely on literature data only.

   In the process-based dynamic models, different microbial decay models are used. The prediction of faecal contamination is generally based on faecal coliform or *E. coli* counts only. The decay model generally uses the first order

kinetic model, proposed by Chick (1908), which integrates a term summarizing all biological aspects of microorganisms (Crane and Moore 1986; Kashefipour *et al*. 2002). This model achieves a reasonable level of accuracy (Salomon and Pommepuy 1990; Pommepuy *et al*. 2006), when using the following equation:

$$N_t/N_0 = 10^{-kt}$$

where N_t: number of bacteria (or viruses) at time t, N_0: number of bacteria (or viruses) at time 0, t: time in days, and k: first order or die-off rate constant, generally estimated by T_{90} (k = $2.303/T_{90}$).

More complex models derived from Chick's laws were proposed to express results obtained with bacterial populations composed of distinct subgroups and to describe non-constant decrease rates or to integrate the effects of parameters such as temperature, sunlight, or salinity (Crane and Moore 1986). A comparison of different log-linear and non-linear models (Gonzalez 1995) revealed that non-linear models adequately describe bacterial survival in the aquatic environment.

For microbial modelling in coastal environments, microbial decay is simulated with varying levels of refinement. Most existing process-based models assume a constant value for T_{90} (for example, 8, 16 and 24 h respectively, in Monteiro *et al*. 1992; Garcia-Barcina *et al*. 2002; and Pommepuy *et al*. 2004). However, variable T_{90} values, obtained according to different parameters such as sunlight intensity (Fiandrino *et al*. 2003); to diurnal variations (Roberts *et al*. 1999b; Kashefipour *et al*. 2002); surface/underlying water (Roberts and Williams 1992); season (Bell *et al*. 1992); or to wet and dry weather (Kashefipour *et al*. 2002) were also used. In a modelling study of nearshore coastal waters (United Kingdom), three different procedures for estimating the decay rate coefficients of faecal coliform according to solar radiation were tested (Kashefipour *et al*. 2006). A time-dependant decay rate for faecal coliforms considering the effects of light, salinity and temperature has been introduced into a 3D model (Hogdins *et al*. 1998). In another model, the decay rate depended on bacterial adaptative responses to the marine environment and on the effect of stress on bacterial physiology (Martin *et al*. 1998; Troussellier *et al*. 1998).

As opposed to faecal bacterial indicators, the potential survival of bacterial or viral pathogen in coastal environments has not been widely investigated. Some T_{90} values, mainly obtained from *in situ* experiments were reviewed in Pommepuy *et al.* (2006). It should be highlighted that viruses are much more persistent in coastal environments than bacteria. Therefore, a T_{90} value of 30 days has been reported in a norovirus modelling study in a French shellfish-farming area (Pommepuy *et al*. 2004).

Microbial contamination in shellfish

To model faecal microbial contamination of shellfish, the relationship between bacterial and/or viral concentrations in the harvesting water and in shellfish flesh has to be specified. Indeed, shellfish filter their food as particles in suspension from surrounding waters and subsequently concentrate and retain potential pathogenic microorganisms present in those waters.

The bioaccumulation and elimination kinetics of enteric bacteria and viruses by bivalve molluscs were found to vary with shellfish species, their physiological status, the types of microorganism and environmental conditions such as temperature and season (Prieur *et al*. 1990; Burkhardt *et al*. 1992; Lees *et al*. 1995; Burkhardt and Calci 2000). In some studies, the concentration of faecal microorganisms in water and in shellfish was determined simultaneously and expressed per 100 ml of water and per 100 g of shellfish flesh and intra-valvular liquid, respectively (Burkhardt and Calci 2000; Shieh *et al*. 2003). For faecal coliform or *E. coli*, the concentration factor between water and shellfish could vary from 1- to about 100-fold greater concentrations in the shellfish (Prieur *et al*. 1990; Burkhart and Calci 2000; Shieh *et al*. 2003) and even more for viruses and phages. For example, in a study including 18 pairing of water/shellfish batches (Gulf of Mexico), the accumulation rate varied from 2 to 146 and 0.2 to 222 for *E. coli* and male-specific coliphages respectively (Shieh *et al*. 2003). From data collected in the United Kingdom, Lees *et al*. (1995) established a relation between geometric means (GMs) of *E. coli* in shellfish and the corresponding GMs in seawater. For the pooled data set, a seawater GM of 100 will give a bioconcentration factor of 5.9 for mussels, and of 2.6 to 6.9 for oysters (Lees *et al*. 1995; European Commission 1996).

Among studies modelling microbial contamination in shellfish in the coastal environment, microbial concentration values in water (predicted at the shellfish sampling sites) were converted to values in shellfish flesh using a constant factor (Pommepuy *et al*. 2004; Riou *et al*. 2007) or using the European Commission (1996) relationships in the study of Tattershall *et al*. (2003). A more complex approach (taking shellfish grazing, filtration rate of oyster and retention efficiency of faecal coliform by oyster into account) was developed by Fiandrino *et al*. (2003).

Microorganisms bound to sediment particles

Enteric bacteria or viruses could be free organisms, possibly associated with dissolved material or bound to organic or inorganic particles, particularly small ones (Gerba 1984; Rao *et al*. 1986; Prieur *et al*. 1990; Auer and Niehaus 1993; Baudart *et al*. 2000). In the study of Auer and Niehaus (1993), 90.5% of faecal

coliforms were found to be associated with particles from 0.45–10 μm. These bound organisms can sediment and possibly be resuspended along with their associated sediment particles during tidal variations, waves, storms, heavy rains, and/or dredging operations (Grimes 1980; Pettibone *et al*. 1996; Coehlo *et al*. 1999). Superficial bed sediments often exhibit a higher microbial contamination than the particles entrained in overlying waters (Martines-Mazanares *et al*. 1992; Irvine and Pettibone 1993; Craig *et al*. 2002) and of bottom sediments (Ferguson *et al*. 1996). This is due in part to the greater bacterial or viral survival within these surface sediments (Le Guyader *et al*. 1991; Chung and Sobsey 1993; Davies *et al*. 1995).

Adsorption to particles, sedimentation of bound faecal microorganisms and resuspension as sediment particles may be important factors affecting bacterial concentrations in water and thus, in shellfish. However, they are not currently specified in modelling systems (Boehm *et al*. 2003). In fact, considering that microorganisms associated with particles and sediment greatly complicate microbial modelling in natural waters, there is a need for the application of a sediment transport model to account for resuspension of faecal microorganisms associated to sediment particles under the action of turbulence and waves. Moreover, many uncertainties still exist concerning microbial contamination of sediment, including the effects of bound microorganisms and their behaviour in coastal environments. At present, the models which aim to describe the effect of sediment transport and in particular, the resuspension of bound faecal microorganisms are not numerous (Steets and Holden 2003; Harris *et al*. 2004; Sanders *et al*. 2005).

16.1.2.4. Model use

Most applications of process-based dynamic models focus on faecal coliforms or *E. coli* in coastal water (Head *et al*. 1992; Falconer and Lin 1997; Roberts 1999; Kashefipour *et al*. 2002; Servais *et al*. 2007). They more rarely address shellfish harvesting areas (Pommepuy and Salomon 1991; Fiandrino *et al*. 2003; Tattersall *et al*. 2003; Riou *et al*. 2007). Two examples of microbial modelling in French shellfish-farming areas are presented below.

Faecal coliform contamination of shellfish in a Mediterranean lagoon with large-scale shellfish farming was modelled during flow events (Fiandrino *et al*. 2003). Simulations were based on bacterial transport and survival and coupled models were forced by the input of bacterial loads from the two main rivers (Vène and Pallas). Different flow types (reference, sudden and constant) were considered and subsequent spatial and temporal bacterial contamination of lagoon surface water and shellfish were estimated. Most of the time, the gradual changes of faecal

coliform abundances in the lagoon were due to biological rather than physical processes. In fact, the ratio of biological decay to physical dilution was found to be of 1 to 50 in a lagoon and of less than 0.002 in an estuary (Salomon and Pommepuy 1990). This indicates that, when the flushing time is very long, biological effects dominate processes determining faecal indicator concentrations. In contrast, in estuaries and coastal areas under the influence of strong tidal currents, physical mechanisms dominate faecal indicator fate and transport because of the high dispersion characteristics produced.

Bacterial contamination of shellfish depends on the receiving area. In the case of the Pallas River area, a simulated sudden input of bacteria led to a short-term (about one day) contamination of shellfish. A constant input of the same amount of bacteria induced a lower level but significant contamination, which was maintained during the entire simulation period (10 days). By contrast, bacterial inputs from the Vène River, led to shellfish contamination only when they were delivered through a flood event. Management of river flow, such as by installation of retention basins on watersheds to regulate the river's hydraulic characteristics might be a way to limit the impact of bacterial contamination of shellfish.

In another study, viral and bacterial contamination in a shellfish-harvesting area was modelled with the aim of simulating the effect of domestic discharges on water quality (Pommepuy *et al.* 2004). The model comprised water concentrations corresponding to 20 days of domestic discharges in the sea during a viral epidemic in the population. The T_{90} values used for *E. coli* and norovirus were 1 and 30 days, respectively. Based on the faecal flux measured from the wastewater treatment plant, average microbial flux was estimated at 3.4×10^9 *E. coli* per second. Viral input was estimated at 10^6 viruses/s based upon the incidence and excretion rate in the population (3%, 60 000 inhabitants). Results from the model calculations were found to be similar to *E. coli* concentrations observed in shellfish. According to this model, physical dilution was sufficient to dilute viral input and limit contamination. Despite the very long viral T_{90} assumed, the calculated viral flux was not sufficient to pollute the area. These calculations were in good agreement with actual viral shellfish contamination data (based on RT-PCR analyses).

16.2 EARLY WARNING SYSTEMS FOR SHELLFISH PRODUCTION SITES

At present, it is not possible to measure bacterial and/or viral concentrations in water or shellfish flesh and to obtain an immediate quantitative result to evaluate

and prevent human health risk. To provide this information, environmental parameters which are proven to be correlated with coliform contamination could be considered. Thus, alternative parameters – salinity or turbidity variations – are used to predict microbial contamination because real-time sensors for these physicochemical parameters are available (Grange 1999; Butler *et al.* 2001; Olyphant 2005; Le Saux *et al.* 2006; Pommepuy *et al.* 2008; Haramoto *et al.* 2007).

Early warning systems, based on modelling, whether statistical or deterministic, were developed or are under development: they aim at obtaining real-time data for risk management. However, few reports were published on these early warning systems. They are used for notification of events, monitoring harvesting closures and for calculation of re-opening time and date for shellfish growing areas. These predictive systems are based on (i) simple relationships between the observed rainfall and faecal microorganism concentrations (Grange 1999) or on (ii) complex models of the dominant mixing and transport processes (EPA 1999; Grange 1999).

The early warning system, proposed by Le Saux *et al.* (2006), is based on real-time observation from control points. The information is immediately sent to a computer which synthesizes the information, if the parameter exceeds defined values, the system moves into an alarm mode (Figure 16.1). Different parameters could be recorded (rainfall, salinity, sewage network key-points, disease epidemics in the contributing community etc) and gathered in a database. Particular events which could cause a deterioration in water quality could thus, be detected and shellfish producers immediately informed (such as an alarm from point 2).

In Europe, an early warning system for shellfish-farming areas is under development (Le Saux *et al.* 2006). The main goal of developing such a system is to predict a potential viral contamination in shellfish taking the following key parameters into account – salinity variations near shellfish at the producing areas; weather conditions in the watershed; and viral disease outbreaks in the local population. Validation of the above parameters is necessary for such systems, involving a comparison of water quality conditions at different sites which could serve as the basis for any water quality advisory notification.

In New Zealand, harvest criteria based on rainfall, river discharge level or salinity as a proxy for faecal coliform contamination have been developed by NIWA in collaboration with the marine farming industry and health authorities (Grange 1999). Data, collected by a series of tipping bucket rain gauges, water level recorders or salinity measuring buoys, are stored at remote stations and downloaded via telemetry or cell phone and compared to pre-determined criteria. The information is automatically faxed to shellfish harvesters and
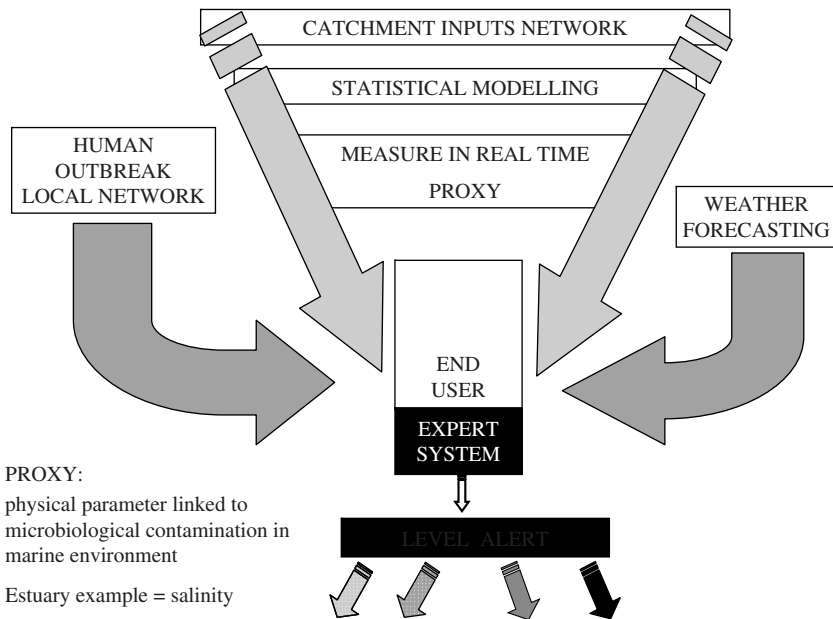
Figure 16.1 Concept of an early warning system.

regulatory autorities, who can also access the data via remote fax machine, often connected to cell phones on vessels (Grange 1999). It is noteworthy that these early warning systems are site-specific as some relevant criteria could be different from one area to another. Such autonomous monitoring networks have also proposed to protect against toxic algal blooms in a fish farm located in a south-eastern Tasmania estuary (Butler *et al*. 2001).

Other parameters, such as real-time data acquired from sewage network key-points and/or disease outbreak monitoring, also provide useful data to be considered in early warning systems. For example, epidemics are recorded in the human population by European Networks (for example in France, sentiweb: www.b3jussieu.fr). These networks could provide information on the occurrence of gastroenteritis in a specific part of the country and, consequently, warn of a possible input of viral pathogen in coastal environment. In the case of sewage, expert systems for monitoring wastewater treatment plants are already available (Punal *et al*. 2002). They could be associated with neural networks to predict wastewater input from sewage treatment plants or agricultural activities. The hydraulic loads and wastewater flow which would reach the river and ultimately the coast can be calculated (Crowther *et al*. 2002; Gamal El-Din and Smith 2002).

16.3 CONCLUSIONS

A broad variety of statistical or process-based models is now available to predict microbial contamination in the coastal environment. The models require information on environmental parameters which can determine microbial contamination at a specific site. They also provide potential for examination of various scenarios by considering, separately or together, different parameters or events that would degrade water quality and thus, contaminate shellfish. Furthermore, a recent study indicates that when combined to epidemiological models, process-based models could predict coastal health risk (Harris *et al.* 2004).

While uncertainties are addressed within process-based models (Harris *et al.* 2004), the most important ones are due to microbial data. In fact, very little precise empirical information exists on microbial behaviour in the environment and when available, they mostly concern faecal indicators and rarely pathogenic bacteria or viruses. Information is sparse on microbial inputs and their decay rates in environmental water, particularly for microorganisms bound to sediment particles, or in shellfish. Collecting this information is necessary to explain shellfish contamination and thus manage the risks.

To improve microbial modelling, different actions have to be developed. First of all, more sensitive, reproducible and standardized methods have to be developed to evaluate microbial concentrations in shellfish, waters and other compartments involved in water degradation – in sewage, catchment systems and sediment. Quantitative molecular methods such as real-time PCR or RT-PCR are now available. If applied to the environment, these methods would give more precise information on the level of non culturable viruses and of viable and culturable or viable but non culturable bacteria in impacted areas (Loisy *et al.* 2005b; Wade *et al.* 2006).

Moreover, technological progress leads us to reconsider research on pathogenic microorganism survival *in situ* and shellfish contamination/decontamination dynamics in the environment and/or during depuration processes. Investigations to improve the knowledge-base of the area would also be of greatest importance to validate coastal models and identify, for example, the role of diffuse sources from animal origins which could carry human pathogenic bacteria or viruses. The development and application of microbial source tracking methods in order to identify sources of faecal pollution could also provide useful additional data (Blanch *et al.* 2004; Gourmelon *et al.* 2007; Santo Domingo and Sadowsky 2007; Santo Domingo *et al.* 2007; Stoeckel and Harwood 2007).

Early warning systems have already been described and first applications presented (Grange 1999; Pommepuy *et al.* 2008). They are predictive tools based on the observation of the most relevant environmental parameters

involved in water degradation, such as salinity variations, sewage or river discharge (EPA 1999; Punal *et al*. 2002). However, further information derived from data recorded by epidemiological survey networks should be included in these systems, together with data on emerging pathogens or presence of related outbreaks in the coastal population leading to more accurate risk assessment and management (Le Saux *et al*. 2006).

In a near future, direct and real-time data on pathogen observations in the environment provided by biosensors, DNA chips (Rose and Grimes 2001; Picup *et al*. 2003; Lee *et al*. 2006; Lazcka *et al*. 2007) or rapid techniques (enzymatic analyses, for example) could be integrated into early warning systems. This would provide an additional safeguard for an efficient consumer protection.

16.4 REFERENCES

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17

Framework for change

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This book, which has been authored by a range of international stakeholders, describes the science and associated societal issues which are driving both concerns and improvement in the management of shellfish harvesting waters world-wide. In this concluding contribution it is not the intention simply to summarize the discussion of preceding chapters. Rather, our remit is to identify:

- the principal causes of concern;
- the sources from which they derive;
- the shellfish species associated with potential disease transmission;
- the transmission pathways and their mitigation potential;
- the key elements of the 'best management practice' in shellfish monitoring programmes; and
- the extent to which the developing regulatory approaches reflecting this understanding could result in risk management and control.

Thus, we seek first to delineate the context for change in the management of shellfish harvesting waters and outline the potentially fruitful directions for future management and research.

It is worth noting that the health risks resulting from shellfish consumption have three drivers. The first is from pathogen contamination of harvesting waters. The second is from toxins derived from algal blooms driven by coastal nutrient enrichment. Both of these elements are caused by anthropogenic inputs to harvesting waters. The third driver is the presence of autochthonous pathogens, particularly *Vibrio* spp., which are a risk in warmer sea waters. There is an historical bias to research and investigations focused on the first driver and, to a large extent, this is reflected in this book. Each of the drivers is covered by, for example, Graczyk *et al.* in chapter 3 of this book, but the management approaches suggested by regulators and governments to date reflect the historical exploitation of temperate northern harvesting areas, where the first driver is the principal concern, and the evidence-base for remediation strategies targeted to the second and third drivers are much less well developed.

17.1 THE MANAGEMENT CHALLENGE

The shellfishery component of fisheries resource utilization comprises less than 10% of the seafood industry (by weight) world-wide and production is dominated by China which produces 68% of world-wide output value. As noted in chapter 1, some 84% of global bivalve production derived is from aquaculture. Thus, many of the environmental waters in which shellfish are grown are commonly in close proximity to anthropogenic pollution sources which will, at times, contain pathogens and nutrients derived from societal fluxes of human sewage and/or livestock waste. The 'outcomes' could be described as 'alarming' with over 85% of Norwalk-like virus (NLV) outbreaks, and all foodborne outbreaks of *Vibrio* spp. and *Plesiomonas shigellosis* in New York, USA between 1980 and 1994, associated with seafood consumption (see chapter 3; Wallace *et al.* 1999). In addition Graczyk *et al.* (chapter 3) note risks from 'naturally occurring' microbial pathogens in warmer nearshore waters such as *Vibrio* spp. which produce gastroenteritis which is much more severe than faecal–oral diarrhetic diseases of generally viral etiology. *V. vulnificus* can also cause infections resulting in fulminant primary septicaemia (often with necrotizing cutaneous lesions) with a high mortality rate. Autochthonous algae also produce toxins when they break down, generally following periods of algal blooms caused by the interaction of anthropogenic nutrient enrichment and natural seasonal patterns of light and temperature changes in coastal waters.

Perhaps of most significance, however, is the fact that shellfish are commonly eaten uncooked particularly where they are sold as a premium product and marketed to stress their 'pristine' environmental source. Added to this is the now extensive, trans-continental shipping of shellfish providing the potential for effective pathogen transfer from areas of high disease endemicity to consumers with potentially low immunocompetence for potentially exotic pathogens.

Thus, there remains the ongoing potential for shellfish consumption both to complete the chain of faecal–oral infection where growing areas are impacted by wastewater and to provide the vector for endemic pathogens in warmer waters. This chain has, in most other food sectors, and in the potable water cycle, been specifically broken through hygiene interventions (for example pasteurisation of milk and chlorination of drinking water). These elements of the shellfish product and its environment conspire to produce a significant and continued health risk to the consuming population which is increasingly seen to have a global dimension. The potential management interventions charted in chapter 1 of this book, do no more than seek to prevent completion of the faecal–oral chain of infection and/or identify appropriate risk indicators for algal and autochthonous pathogen risks, thus, to limit illness in the consumer population.

The central management challenge and many of the approaches available for achieving safe shellfish for human consumption have remained unchanged since the pre-historical beginnings of this resource utilization. However, the scientific and regulatory toolbox available to prevent disease transmission is growing apace with very significant developments coming from a diverse community of stakeholders.

17.2 NEW TOOLS AND APPROACHES

Perhaps the most significant element in the regulatory toolbox is the growing conceptual understanding of the complex interacting systems which produce the apparently random and chaotic changes in microbial and toxin levels in shellfish harvesting waters. This understanding principally derives from recent 'drainage-basin' paradigms adopted by environmental regulators first in North America with the Clean Water Act and subsequently in Europe with the Water Framework Directive (Anon 2000). These instruments replaced historical approaches based on equal regulation of individual discharges throughout a catchment. This produced equal treatment for dischargers but resulted in environmental and resource impairment where multiple 'consented' discharges could easily overwhelm the assimilative capacity of specific river reaches or receiving water bodies. The new paradigm focuses on the ecological and resource use requirements of the receiving water and all upstream discharges

are managed in an integrated manner to prevent resource 'impairment' at the point of use.

Implementation of this developing regulatory model implies knowledge of:

i. the contribution of the various pollutant sources, in the case of shellfish harvesting water, principally quantitative microbial source apportionment;

ii. the spatial pattern of multiple inputs from within the contributing catchment where point and diffuse elements of particularly microbial pollutants may derive from animal and human sources, in addition to endemic pathogens;

iii. the fate and transport of pollutants as they pass through the complex set of hydrological, pedalogical and geological/sedimentary compartments within the catchment and nearshore systems; and finally,

iv. the processes driving the transport mechanisms operative within and between these complex interacting compartments which produce the highly episodic and seasonal variability in microbial and nutrient concentrations impacting on shellfish harvesting areas.

Detailed deterministic process understanding of these complex interacting systems is not, at present, available for catchment and nearshore microbial dynamics. However, the introduction of the new holistic regulatory approach has sparked a series of scientific initiatives which taken together are advancing knowledge in this area rapidly.

Initial responses designed to accommodate these holistic catchment concepts within a series of practical steps which could be taken on the ground by shellfish regulators can be seen in the design of a 'sanitary survey' as explained for New Zealand waters by Busby (chapter 13) and for Australian waters in Anon (2004). These represent the first regulatory attempts to recognize the complexity implied in bullets i to iv above whilst designing clearly defined stages in problem scoping through an initial sanitary survey and progress monitoring through annual reviews. Importantly, the extreme variability in faecal indicator concentrations and the periods of maximum risk are explicitly recognized in the sampling protocol for regulatory monitoring which requires microbial data acquisition to be undertaken during periods of adverse weather conditions.

Thus, for example, the New Zealand regulations are specifically designed to accommodate the spatial and temporal complexity through sanitary survey and temporally targeted sampling. This provides an innovative and intelligent, but practical, means of regulating the complexity implied in i to iv which is also reflected in United States regulations (chapter 7).

Further improvement to risk control beyond this immediate regulatory approach would require better process understanding of the complex catchment and coastal compartments outlined in i to iv. It is perhaps in this area where the impacts of the new regulatory paradigm will prove most significant in years to come.

Already new approaches have been developed to distinguish between animal and human microbial pollution using a suite of methods which have become known as microbial source tracking (MST). Santo Domingo and Edge (chapter 5) outline the application of these approaches to shellfish harvesting waters and chart a path to deciding which of the many approaches is most appropriate for specific situations. Delineation of animal and human sources impacting on specific growing areas is important because of the prominence of human-specific pathogenic viruses in historical shellfish-associated disease outbreaks (chapter 3), although zoonotic pathogens cannot be ignored (Macrae *et al.* 2005; Gourmelon *et al.* 2006; Levesque *et al.* 2006; Downey and Graczyk 2007; Graczyk *et al.* 2007; Leoni *et al.* 2007; Schets *et al.* 2007). There are major research efforts world-wide producing rapid advances in MST which is being used to inform regulatory decisions and practice often using MST approaches developed by the regulators themselves (Gawler *et al.* 2007). It should be noted that MST approaches currently afford only 'qualitative' information on likely contributing species (commonly either ruminant and/or human) and cannot provide precise quantification of the different contributing animal species to microbial loadings above a specific harvesting area (Stapleton *et al.* 2007). However, considerable potential exists for further development in this area which, if it could provide quantitative source apportionment, would prove extremely valuable in informing a sanitary profiling exercise.

Process and black box modelling of microbial concentrations in catchment and nearshore systems is the second area of significant investigation which has received impetus from the new regulatory paradigm. Catchment microbial modelling is a new and emerging discipline with some operationally useful black box models (Kay *et al.* 2005) which lack the process components to inform management decisions on appropriate control strategies and growing efforts to develop process-based, deterministic catchment models (Jamieson *et al.* 2003; Jamieson *et al.* 2004a; Jamieson *et al.* 2004b; Jamieson *et al.* 2005a; Jamieson *et al.* 2005b; Ferguson 2005; Ferguson *et al.* 2003a; Ferguson *et al.* 2003b; Oliver *et al.* 2005a; Oliver *et al.* 2005b; Oliver *et al.* 2006; Oliver *et al.* 2007). As this research area develops, operational microbial deterministic models will become available to the regulatory community and will provide predictive capacity for the implementation of both sanitary surveys and mitigation measures which are identified for priority action to achieve regulatory compliance (Kanso *et al.* 2005; Kay *et al.* 2007; Qian and Reckhow 2007).

Nearshore hydrodynamic and water quality modelling of microbial concentrations is certainly a more mature science with well established methods and approaches (chapter 16). Parallel statistical modelling developed in response to the WHO Guidelines for Safe Recreational Water Environments (WHO 2003) is also discussed and under development in several countries (Crowther *et al.* 2001; USGS 2003, 2006, 2006). Perhaps the largest significant weakness in both nearshore modelling approaches is the lack of good calibration data which characterises the dynamic and highly episodic patterns of microbial concentration experienced in nearshore waters. It is also apparent that, often, the model developers with high level skills in mathematics, hydraulics and environmental physics do not fully appreciate the nature of microbiological data and specifically the inherent imprecision in microbial enumerations which are used to provide the often sparse calibration data for complex numerical modelling exercises. Without such an appreciation, which can only derive from inter-disciplinary working, spurious precision in predicted values is too easily assumed which can lead to ill-informed and inappropriate expenditures and/or choice of harvesting areas. In part, this may derive from the different disciplinary perspectives of the hydrological modelling and microbiological communities, the former concerned with accurate hydrological flux prediction and the latter with characterisation of peak risk episodes. Thus the definition and estimation of imprecision needs integrated attention of these two communities. It is interesting to note that Gourmelon *et al.* (chapter 16) explain the use of telemetric real-time data acquisition for warning trigger activation rather than assuming the predictive reliability of deterministic modelling in this area.

As the combined catchment and coastal modelling agendas converge and the modelling tools become more precise and truly 'predictive', they will provide reliable predictive tools able to inform and drive the future management, regulation and remediation of impaired harvesting areas.

17.3 THE DEVELOPING AGENDA

Regulation and management of shellfish harvesting waters is at a challenging and very fluid stage because there is a growing understanding of the processes driving the physical systems operative within linked catchment and nearshore environments. These produce short periods of high risk to microbial shellfish flesh 'compliance', and associated health risk caused by elevated microbial concentrations caused by episodic transport processes driving concurrent fluxes from the sewerage system (Kay *et al.* 2008) and/or agricultural diffuse sources (Wilkinson *et al.* 2006; Kay *et al.* 2008). Traditional regulatory systems involving monitoring regimes with regular sampling intervals (or even pseudo-random

sampling within a period, such as a month) are unlikely to characterize the brief periods of peak risk to the consumer. Indeed, monitoring at regular time intervals is systematically biased not to characterize episodic peak risk periods. The New Zealand approach, involving targeted monitoring during peak risk periods, can be seen as an initial recognition of this variability and it seeks to ensure public health protection through the adjustment of sample collection. However, this implies resource availability for pro-active, opportunistic sampling and analysis by the regulatory community and/or reliable predictive modelling to underpin regulatory use of surrogates such as rainfall.

The nature of microbiological data capture will always introduce an information lag between sample acquisition and management information being available to the regulatory and public health communities. Real-time measurement of surrogate variables, such as salinity and turbidity, linked to parallel telemetric data transmission is one approach available (chapter 16) to provide near-real-time management information on episodic risk. However, this does not involve actual microbiological information. Acquisition of this type of microbiological data has formed a central component of the US EPA's research agenda for both bathing waters (Wade *et al.* 2003; Haugland *et al.* 2005; Wade *et al.* 2006) and shellfish harvesting areas (chapter 3). In this work, a considerable effort has been devoted to the development of rapid methods (generally quantitative polymerase chain reaction-based) of faecal indicator enumeration, rather than direct virus and other pathogen quantification which has proven problematical in both water and flesh matrices (Croci *et al.* 2007; Schultz *et al.* 2007). However, there are likely to be significant developments in near-real-time direct pathogen enumeration as well as enhanced indicator quantification in the next few years (Brands *et al.* 2005; Rizvi *et al.* 2006; David *et al.* 2007; Gabrieli *et al.* 2007; Maekawa *et al.* 2007; Phan *et al.* 2007; Saitoh *et al.* 2007; Schultz *et al.* 2007) which will shorten the current lag in the availability of management information from a few days to a few hours.

Acquisition of any 'spot' determination from a sample of the environment will always be a snapshot in time and very many such 'snapshots' are required to characterize the highly dynamic microbial risk pattern evident in shellfish harvesting waters. It is here that accurate predictive microbial modelling can make its major contribution. The potential exists to predict the level and duration of the key peak risk episodes which has two principal advantages for the regulatory community. First, it can guide sampling programmes to the most appropriate periods of data acquisition and suggest appropriate periods when harvesting would be inappropriate for defined treatment interventions (chapter 9). Second, truly deterministic and process-based modelling tools can guide remediation strategies through the understanding afforded of the range of

interventions covering point source disinfection, through agricultural best management practices (BMP) at the field scale (chapter 15). Realistically, the availability of such tested tools are further into the future but the experience of other related modelling communities addressing, for example, catchment derived nutrient fluxes, offers considerable encouragement to the shellfish regulator.

17.4 CONCLUSIONS – CURRENT STATE OF PLAY AND WAY FORWARD

Whilst speculation on potential developments is interesting and important, we suggested a series of questions above which this chapter now addresses. The most intensively researched and managed driver as defined above remains the enteric pathogens which are concentrated in shellfish flesh and cause significant illness outbreaks world-wide. They derive mainly from human sewage disposal, although zoonotic pathogens from agricultural activity and autochthonous 'natural' pathogens cannot be discounted. Filter-feeding bivalve molluscs are the principal species of concern particularly where they are lightly cooked or consumed raw. The transmission pathways involve complex catchment to coastal systems and mitigation involves an integrated spectrum of practices covering both point source control of treated effluent discharges and agricultural BMPs to attenuate flows from diffuse agricultural sources.

Best practice in current monitoring programmes is increasingly seeking to target peak risk episodes and intelligently adjust sampling regimes to acquire data during such periods. However, there will always be compromises where sampling resource is limited and the regulator must seek to characterize pathogen risk from a relatively small number of samples in which faecal indicators are commonly enumerated. The emerging tools of:

- rapid methods for near-real-time indicator and pathogen enumeration;
- real-time monitoring of surrogate parameters and telemetric data transmission; and
- predictive modelling using both statistical (black box) and deterministic (white box) approaches offer potential for:
  - (1) more appropriately targeted regulatory sampling;
  - (2) continuous risk assessment particularly in periods where physical samples have not been collected; and
  - (3) predicting the impacts of different remediation and BMP scenarios implemented at the catchment scale.

Thus, there is considerable immediate potential for better regulation and improved public health with significant additional gains likely in this area as the developing tools become available to underpin a sustainable use of shellfish resources world-wide. It would therefore seem timely, fitting and logical to begin to realise that potential without delay to achieve those health gains in a consistent and collaborative way.

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