

# **Review of Depuration and its Role in Shellfish Quality Assurance**

K.L. Jackson  
NSW Shellfish Quality Assurance Program  
Locked Bag 247  
PYRMONT NSW 2009  
AUSTRALIA

and

D.M. Ogburn  
NSW Fisheries  
Port Stephens Research Centre  
Taylors Beach Road  
TAYLORS BEACH NSW 2301  
AUSTRALIA



**NEW SOUTH WALES  
SHELLFISH QUALITY  
ASSURANCE PROGRAM**



**FRDC Project No. 96/355**

1999

NSW Fisheries Final Report Series

No. 13

ISSN 1440-3544



**Review of Depuration and its Role in Shellfish  
Quality Assurance**

K.L. Jackson  
NSW Shellfish Quality Assurance Program  
Locked Bag 247  
PYRMONT NSW 2009  
AUSTRALIA

and

D.M. Ogburn  
NSW Fisheries  
Port Stephens Research Centre  
Taylors Beach Road  
TAYLORS BEACH NSW 2301  
AUSTRALIA

**FRDC Project No. 96/355**

1999

NSW Fisheries Final Report Series

No. 13

ISSN 1440-3544



## TABLE OF CONTENTS

|   |             |
|---|-------------|
| <b>Table of Contents</b>  | <b>i</b>    |
| <b>Non Technical Summary</b>  | <b>iii</b>  |
| <b>Recommendations</b>  | <b>v</b>    |
| <b>Background</b>   | <b>viii</b> |
| <b>Need</b>   | <b>ix</b>   |
| <b>Objectives</b>   | <b>x</b>    |
| <b>Benefits</b>   | <b>x</b>    |
| <br>  |             |
| <b>1. Introduction</b>  | <b>1</b>    |
| 1.1. History of Depuration  | 1           |
| 1.2. Shellfish and Infectious Disease   | 2           |
| 1.3. Shellfish-Associated Disease Outbreaks   | 3           |
| 1.4. Shellfish Sanitation Strategies and Disease Outbreaks                          | 4           |
| Figure 1: Major NSW oyster producing estuaries                                      | 8           |
| <br>  |             |
| <b>2. Pathogenic Organisms Implicated in Shellfish Associated Disease Outbreaks</b> | <b>9</b>    |
| 2.1. Viral Agents   | 9           |
| 2.2. Bacterial Agents   | 10          |
| 2.2.1 <i>Vibrio</i> spp.  | 10          |
| 2.2.2. Other Infectious Bacterial Agents  | 10          |
| 2.3. Protozoan Parasites  | 11          |
| 2.4. Heavy Metals, Pesticides and Chemical Toxins                                   | 11          |
| 2.5. Summary of Infectious Disease Associated with Shellfish                        | 12          |
| <br>  |             |
| <b>3. Overview of Oyster Physiology</b>   | <b>13</b>   |
| 3.1. Gastrointestinal Tract and Feeding Activity                                    | 13          |
| 3.2. Environmental Requirements   | 14          |
| 3.3. Summary  | 15          |
| <br>  |             |
| <b>4. Overview of Shellfish Culture and Modes of Contamination</b>                  | <b>16</b>   |
| 4.1. Growout  | 16          |
| 4.2. Harvesting   | 16          |
| 4.3. Depuration   | 17          |
| 4.4. Transport  | 18          |
| 4.5. Processing   | 18          |
| 4.6. Consumption  | 19          |
| 4.7. Summary of Factors Affecting the Sanitary Quality of Shellfish                 | 19          |
| Figure 2: Summary of Factors Which May Affect the Sanitary Quality of Shellfish     | 20          |
| <br>  |             |
| <b>5. Shellfish Sanitation Programs</b>   | <b>21</b>   |
| 5.1. Classification of Shellfish Growing Areas                                      | 21          |
| 5.2. HACCP-Based Shellfish Quality Assurance Programs                               | 24          |
| <br>  |             |
| <b>6. Microbes and Biotoxins in the Estuarine Environment</b>                       | <b>26</b>   |
| 6.1. Detection of Microbes and Biotoxins  | 26          |
| 6.1.1. Detection Methods Based on Culture of the Organism                           | 26          |
| 6.1.1.1. Faecal Coliforms Including <i>Escherichia coli</i>                         | 26          |
| 6.1.1.2. <i>Vibrio</i> spp.   | 27          |
| 6.1.2. Immunological Detection Methods  | 28          |
| 6.1.3. Genetic Techniques for Identifying Pathogens                                 | 29          |

|  |           |
|--|-----------|
| 6.1.4. Detection of Viral Agents   | 29        |
| 6.1.5. Detection of Biotoxins  | 31        |
| 6.2. Microbes in the Environment   | 31        |
| 6.2.1. Faecal Bacteria in the Environment and the Concept of Indicator Organisms | 32        |
| 6.2.2. Vibrio Species of Bacteria in the Environment                             | 34        |
| 6.2.3. Viral Agents in the Environment   | 35        |
| 6.2.4. Summary of Microbes in the Environment                                    | 36        |
| 6.2.5. Microbial Contamination of Shellfish Under Laboratory Conditions          | 37        |
| 6.2.5.1. Critique of Experimental Design of Laboratory Studies                   | 38        |
| <b>7. Depuration Research</b>  | <b>40</b> |
| 7.1. Principal Types of Depuration Systems                                       | 40        |
| 7.2. Methods of Water Disinfection   | 40        |
| 7.2.1. Ultraviolet Disinfection  | 40        |
| 7.2.1.1. Variables Influencing the Efficacy of UV Disinfection                   | 41        |
| 7.2.1.2. Efficacy of UV Radiation  | 42        |
| 7.2.2. Ozone   | 42        |
| 7.2.3. Chlorine  | 43        |
| 7.2.4. Iodine  | 44        |
| 7.2.5. Summary of Disinfection   | 45        |
| 7.3. Temperature and Salinity of Depuration Water                                | 45        |
| 7.4. Turbidity   | 48        |
| 7.4.1. Turbidity Standards   | 49        |
| 7.5. The Effect of Initial Pathogen Load on the Efficacy of Depuration           | 50        |
| 7.6. The Effect of Tissue Distribution of Microbes on Depuration Efficacy        | 51        |
| 7.7. Depuration of Different Microbes  | 51        |
| 7.7.1. Bacterial Agents  | 52        |
| 7.7.2. Viral Agents  | 56        |
| 7.7.3. Comparative Removal Rates of Viral and Bacterial Agents During Depuration | 57        |
| 7.7.4. Biotoxins   | 57        |
| 7.8. Summary of Depuration Efficacy  | 58        |
| <b>References</b>  | <b>60</b> |
| <b>Appendix 1</b>  | <b>89</b> |

## NON-TECHNICAL SUMMARY

|               |   |
|---------------|---|
| <b>96/355</b> | <b>Review of Depuration and its role in Shellfish Quality Assurance</b> |
|---------------|---|

**Principal Investigators:** Dr K. Jackson  
**Address:** Locked Bag 247  
PYRMONT NSW 2009  
Telephone (02) 9566 7843 Fax: (02) 9518 4895

Mr D. Ogburn  
NSW Fisheries  
Taylors Beach Road  
TAYLORS BEACH NSW 2316  
Telephone (02) 4980 4919 Fax: (02) 4981 9074

### Objectives:

1. To conduct a desktop review of R&D in shellfish purification technology that may be pertinent to the NSW Oyster Industry and the aims and objectives of the NSW Shellfish Quality Assurance Program (NSW SQAP). The main objective of the NSW SQAP is to ensure that shellfish are taken from estuarine waters to be sold for human consumption only if the shellfish and shellfish culture waters meet specified standards.
2. Provide advice to industry, the NSW Shellfish Quality Assurance Committee and the Oyster Research Advisory Committee on the status of shellfish purification R&D to enable industry to make decisions on future directions in purification technology investments and identify areas requiring further R&D in purification.
3. Provide a summary of the current “state of the art” and limitations of purification technology and R&D accomplishments to date.
4. Identify promising and likely future directions in matters relating to purification, particularly in terms of efficiency and issues such as viruses and biotoxins and usefulness of rapid detection techniques.
5. Make recommendations on HACCP quality assurance programs including the use of water quality monitoring programs as an integral part of the overall purification procedure.

Depuration in the context of this report can be defined as the process by which harvested shellfish are placed in land based plants containing clean estuarine water to permit the purging of their gastrointestinal contents under controlled conditions. Depuration does not include the practice of relaying shellfish to clean estuarine waters for long periods to promote self cleansing.

Shellfish (defined as bivalve molluscs for the purposes of this review) filter large volumes of water and trap particulate matter and dissolved substances suspended in the water as a

source of food. Consequently, if the water in which they are grown is polluted, then the shellfish may concentrate microbes or chemicals which may be injurious to the consumer. Because shellfish are often consumed raw or (slightly cooked) and whole, (including their gastrointestinal tract), they are generally classified as a high-risk food group by health authorities worldwide.

Shellfish are depurated in order to reduce the likelihood of transmitting infectious agents to consumers. Depuration has been demonstrated to successfully reduce to low levels the number of bacterial and some viral agents in moderately polluted shellfish. The effectiveness of the depuration process is dependent on a number of variables including the health status of the shellfish, environmental parameters within the depuration plant (salinity, temperature, turbidity), the type of pathogen, and level of contamination.

Depuration has been practiced around the world since early this century. In 1978 the practice was formally introduced in NSW as a response to a food poisoning outbreak involving over 2000 cases of viral gastroenteritis, which was attributed to oysters farmed in the Georges River. Depuration of all oysters harvested in NSW became a statutory requirement in 1983.

After reviewing the literature and consulting industry and relevant experts, two areas of contention have been identified with the practice of depuration in NSW and perhaps elsewhere in the world. The first issue relates to operational parameters and regulation of the process, the second appears to be a simple failure by sections of industry and regulatory authorities to appreciate that depuration alone does not ensure shellfish food safety.

At the time depuration was introduced in NSW, environmental factors which affect the rate of depuration (e.g. temperature, salinity and turbidity) were identified as critical control points in the process and as such required further investigation (Fleet, 1978). Despite the commercial practice of depuration for almost 20 years in NSW many of these questions remain unanswered. The absence of this information has resulted in the adoption of arbitrary environmental parameters for the operation of depuration plants. It appears that these arbitrary standards have not been capable of providing an adequate guide to conditions which will promote optimal oyster function and consequently ensure efficient depuration. This deficiency is illustrated by the continued difficulties experienced by plant operators in successfully depurating product for the required time period whilst avoiding oyster spawning. Appropriate, scientifically valid operating parameters need to be determined for depuration plants throughout NSW. This fundamental information is of paramount importance to permit the efficient, successful and cost effective operation of depuration facilities.

The number of depuration plants currently registered in NSW, relative to shellfish production, is much higher than in most other countries. Regulation and assessment of the performance of this large number of depuration facilities is logistically difficult and a drain on the limited resources of the regulatory agency, NSW Health. In order to promote economic efficiency and ease regulatory supervision the number of depuration plants in NSW should be reduced and independent audit procedures implemented. Larger, professionally managed plants would provide an economy of scale encouraging the development and adherence to industry best practice, whilst being more amenable to regular audit.

Continuing food poisoning outbreaks in NSW despite mandatory depuration demonstrates that the process alone is not sufficient to ensure the food safety of shellfish product. It is now recognised that the success of depuration is intimately associated with an understanding of the farming environment and an appropriate monitoring program to assess growing water and shellfish quality. Depuration is a process which will confer a level of additional food safety assurance to shellfish harvested in accordance with stringent protocols, from areas which may be subject to intermittent pollution. Depuration will not render shellfish grown in heavily polluted waters a safe product. A sanitary survey and continuing bacteriological monitoring program are necessary to identify areas which are inappropriate for harvest under any circumstances and those where depuration will be a useful adjunct to quality assurance.

Viral agents are potentially the major cause of foodborne illness and in recent times have been implicated in several large shellfish associated food poisoning outbreaks. Investigation of such outbreaks has often demonstrated sewage contamination of the shellfish growing area, and human enteric viruses such as Norwalk virus and hepatitis A are common aetiological agents. Standardised molecular techniques for the detection of human enteric viral agents in shellfish and research to ascertain the effectiveness of depuration in the elimination of such agents, would assist in the development of improved shellfish sanitary control procedures and consequently benefit the shellfish industry and consumers.

Apart from the continuing need to eliminate pollution of waterways, additional procedures which would promote improved quality assurance procedures in the shellfish industry include the implementation of HACCP based food safety plans by all producers, formal training of shellfish producers in sanitary control measures, and the development of a labelling system so that product can be reliably identified from the farm to the consumer.

**Keywords:**

depuration, shellfish, quality, viruses, bacteria, HACCP.

**Recommendations****a. Short term**

Appropriate, scientifically valid operating parameters (e.g. temperature and salinity) which optimise shellfish physiological processes to permit effective depuration, whilst preventing or reducing the incidence of spawning, need to be determined for depuration plants in NSW.

1. Research should be carried out to determine the level at which suspended particles (that cause turbidity);
  - interfere with UV light transmission in depuration plants
  - interfere with shellfish filtration and depuration effectiveness.
2. The initial level of indicator organisms and other contaminants present in shellfish prior to depuration is a critical point. In NSW further evaluation of these levels is required

and must take into account ambient water quality and the potential risk of both point and non-point source pollution in the estuary, as identified by a sanitary survey. This process should also consider the effectiveness of individual depuration plants in respect of plant design, construction and process variables such as environmental parameters.

3. Strategies which will enhance the effectiveness of UV disinfection should be examined. These may involve the use of additional steps in the preparation and disinfection process, such as filtration.
4. Independent audits of depuration facilities and their performance should be implemented.
5. Strategies which aim to reduce the number of depuration facilities in NSW should be promoted.
6. All shellfish producers should implement a HACCP based food safety plan. A model program could be provided and modified to reflect the individual farm business.
7. Industry should introduce a formal accredited training program in sanitary shellfish production, depuration plant operation and processing. Such training should be a requirement for all depuration plant permit holders.

#### **b. Long term**

1. NSW estuaries should be classified in a manner consistent with the Australian Shellfish Sanitation Program (ASSP) and depuration practiced only in those areas where appropriate water and oyster quality is not consistently available, as specified by the ASSP.
2. Regulatory agencies should seek to develop cost effective techniques for monitoring hepatitis A and Norwalk virus that can be implemented when necessary or in areas which have been identified, on the basis of a sanitary survey, as high risk. Sentinel oysters located at strategic sample sites, e.g. downstream from sewage treatment plant discharge points, may provide potentially useful information regarding the performance of sewage treatment plants and their contribution to estuary contamination.
3. Research is required to develop standardised molecular techniques for the detection of human enteric viral agents in shellfish.
4. Further experimentation needs to be done to ascertain the efficacy of depuration for the elimination of viral agents from Sydney rock oysters and Pacific oysters. These studies should at least consider:
  - the nature of the contamination (natural versus artificial, and initial load).
  - the tissue distribution of the contaminants before and after depuration.
  - the elimination of viral agents from naturally contaminated oysters.

- environmental parameters during depuration, eg turbidity, temperature and salinity.
  - the environment to which the oysters had been previously acclimatised.
  - a suitable technique for measuring the activity of oysters.
5. A strategy for the assessment of marine biotoxin risk should be developed. An early warning system should be implemented in areas considered at risk and an algal bloom response strategy developed.
  6. A labelling system needs to be developed so that produce can be reliably identified from the farm to the consumer.

## Background

In 1978 a food poisoning outbreak involving over 2000 clinical cases of viral gastroenteritis was attributed to the consumption of contaminated oysters farmed in the Georges River, NSW. The outbreak was the result of heavy rainfall that was presumed to have caused sewage overflows into the harvest area (Murphy *et al.*, 1979). This incident undermined consumer confidence in NSW-produced oysters and led to the introduction of compulsory depuration throughout the state. Further oyster associated food poisoning outbreaks in the 1980's and 1990's demonstrated that the implementation of depuration alone was not sufficient to adequately address the problem of shellfish food safety in NSW. The inadequacy of this strategy was again highlighted in 1997 with an outbreak of hepatitis A which was associated with oysters harvested from Wallis Lake, the largest oyster producing estuary in NSW.

The NSW oyster industry has suffered adverse publicity and a loss of consumer confidence following each of these events and this has been reflected in terms of reduced sales and profits and an impediment to increase the price of the product. Competing foods in general, and interstate and New Zealand oysters in particular, have gained improved market share as public perceptions of NSW oysters have declined. The reduction in sales and profits, and retarded prices have also led to a sharp decline in investment confidence, depriving the industry of development capital, reducing farm values and contributing to some bankruptcies.

### Shellfish Depuration and Quality Assurance History in NSW

Depuration was formally introduced in NSW in 1978 as a response to the food poisoning outbreak associated with oysters harvested from the Georges River. Subsequently depuration was progressively introduced to estuaries identified as high risk, and by 1983 depuration of all shellfish harvested in NSW became a statutory requirement, regardless of the sanitary status of the estuary from where the shellfish were harvested.

In NSW, water used in the depuration process is usually disinfected by exposure to ultraviolet light (UV). The UV technology employed is generally based on that used in the United Kingdom, although one large plant using ozone for the disinfection process, and using French derived technology and techniques has been constructed. At the time depuration was introduced in NSW it was recognised that environmental factors which impact on the depuration process (eg temperature, salinity and turbidity) required investigation (Fleet, 1978). Although considerable research was undertaken as the depuration process was initially adapted for local conditions, particularly in the Georges River, preliminary research to determine optimal operational parameters for depuration throughout NSW was never completed. Depuration has been practiced for almost 20 years in NSW, however operating parameters which permit effective depuration have not been conclusively determined for all geographic areas.

The first formal Shellfish Quality Assurance Program (SQAP) for shellfish produced in NSW was introduced in 1990 by oyster farmers on Georges River in an attempt to overcome continuing problems of meeting statutory microbiological requirements, and to restore consumer confidence in locally produced oysters. The main elements of this SQAP

was depuration combined with continual microbiological testing and cessation of harvest during times of high rainfall or detected pollution. The Georges River SQAP was a voluntary program and was a precursor to the development of a state-wide compulsory SQAP.

The NSW Shellfish Quality Assurance Program (NSW SQAP) was implemented in December 1997. This compulsory program is primarily funded by industry and incorporates a strategy of estuarine water and shellfish monitoring based on a shoreline survey to identify potential pollution sources, in addition to compulsory depuration. The NSW SQAP is currently restricted to commercially farmed oysters, and at this early stage, an appraisal of its impact on shellfish safety is not possible. The program is scheduled for an independent audit after 12 months operation and its success will be largely gauged by the incidence of disease linked to the consumption of oysters produced in NSW.

Despite the introduction of the compulsory NSW Shellfish Quality Assurance Program and the continued legislative requirement for depuration, there is yet to be a comprehensive investigation of the potential limitations of depuration as practiced in NSW and an assessment of techniques which may improve cost effectiveness and overall product quality.

## **Need**

The NSW oyster industry and the Australian shellfish industry at large can ill afford further food poisoning outbreaks related to the consumption of contaminated shellfish product. The 1997 Wallis Lake hepatitis A outbreak aptly demonstrated the devastating impact and far reaching implications such outbreaks have on the seafood sector and the community generally.

The development of a broader understanding of the limitations of depuration and the development of strategies to enhance and render the current process more effective, would make an important contribution to public health as well as the image, confidence and ultimate financial well being of the shellfish industry.

NSW Fisheries in conjunction with the Oyster Research Advisory Committee (ORAC) coordinated the preparation of a NSW Oyster Industry Research and Development Strategic Plan, prepared by Ruello and Associates, which was submitted to FRDC in June 1996. The report, through questionnaires and consultation with industry, identifies one of the major current R&D priorities in the NSW oyster industry as assessment of the legislative requirement for purification and where the process is identified as necessary, the development of effective depuration methods which provide for a high level of consumer safety. Issues raised included the effectiveness of purification; the potential for developing more efficient processes for achieving the goal of producing oysters safe for human consumption and possible variations to the existing purification requirements that may be more practical, effective and economic. The report recommended that one of the urgent priorities was for an academic expert in the relevant field of work to undertake a desktop review of the literature in relation to shellfish purification.

There has been considerable work undertaken in the field of depuration in Australia and abroad since legislation was introduced mandating the use of the process in NSW. It is a

complex field and much of the literature and views held by both industry and researchers appears to be either overlapping, conflicting or making claims that may not be fully founded. An appraisal of the current status and the practical limitations of depuration, and recommendations on areas worth pursuing in shellfish depuration technology development is timely if not overdue. Such a review will not only benefit the NSW shellfish industry but has potential application for emerging shellfish farming industries in other states.

## **Objectives**

1. To conduct a desktop review of R&D in shellfish purification technology that is pertinent to the NSW oyster industry and the aims and objectives of the NSW Shellfish Quality Assurance Program. The main objective of the NSW SQAP is to ensure that shellfish are taken from estuarine waters to be sold for human consumption only if the shellfish and shellfish culture waters meet specified standards.
2. Provide advice to industry, the NSW Shellfish Quality Assurance Committee and the Oyster Research Advisory Committee on the status of shellfish purification R&D to enable industry to make decisions on future directions in purification technology investments and identify areas requiring further R&D in purification.
3. Provide a summary of the current “state of the art” and limitations of purification technology and R&D accomplishments to date.
4. Identify promising and likely future directions in matters relating to purification, particularly in terms of efficiency and issues such as viruses and biotoxins and the usefulness of rapid detection techniques.
5. Make recommendations on HACCP quality assurance programs including the use of water quality monitoring programs as an integral part of the overall purification procedure.

## **Methods:**

The project encompassed a review of all available literature relevant to shellfish depuration technology, research and development. Database searches included the National Seafood Centre and the CSIRO Food Technology Section.

Industry Associations (Oyster Farmers’ Association of NSW and the NSW Farmers’ Oyster Section), members of the NSW Shellfish Quality Assurance Committee and Oyster Research Advisory Committee, representatives from NSW Fisheries (Mr Andrew Derwent) and NSW Health (Mr Phillip Bird), as well as recognised experts in the field of depuration, Dr Peter Ayres and Professor Graham Fleet, were consulted in relation to this project. Key site visits of depuration facilities located in NSW were also undertaken during the study.

## **Benefits**

The ability of current depuration techniques to adequately assure the safety of shellfish for human consumption has been questioned, especially when used in isolation and in relation to viral pathogens. It is now recognised that the success of depuration is ultimately dependant on control of the process, understanding of the harvest environment and

monitoring of shellfish growing waters and shellfish product. A considerable amount of work has been completed around the world since depuration was introduced in NSW. A review of this available information is necessary and has been identified as an urgent priority in the recently completed NSW Oyster Industry R & D strategic plan.

As depuration is a statutory requirement in NSW, a review of the current status of depuration in NSW and the identification of methods which may enhance the effectiveness of the process will be of immense benefit to the NSW oyster industry. In addition, any improvements in the management or operation of depuration will enhance the NSW SQAP and provide greater assurance for consumers of NSW produced shellfish.

The benefits of this project are:

1. Production of the review document
2. Discussion and comparison of the use of depuration around the world as a component of shellfish quality assurance programs.
3. Recommendations to improve the effectiveness of depuration as practiced in NSW.
4. Improvement of the quality assurance process for NSW produced shellfish.
5. Implementation of the recommendations from this project will lead to greater consumer safety.
6. Improved confidence and economic stability in the NSW oyster industry.

Industry, resource managers, research organisations, education institutions and administering agencies will be informed of the results of the report and its recommendations by a combination of:

1. Publication of articles in relevant newsletters and journals.
2. Consultation with the industry and other government agencies and user/consumer groups in the form of workshops, seminars, articles and meetings.
3. Utilisation in the development of NSW Shellfish HACCP program; use in the TAFE funded education program to be run by the NSW SQAC for NSW oyster farmers and processors ie. "Quality Assurance in the NSW Oyster Industry"; and availability to AQIS and the Australian Shellfish Quality Assurance Advisory Committee for use in determining standards for the Australian Shellfish Sanitation Control Program.



## 1. Introduction

### 1.1. History of Depuration

Depuration in the context of this report, can be defined as the process by which harvested shellfish are placed in a land-based plant containing clean estuarine water, to permit the purging of their gastrointestinal contents under controlled conditions. Depuration does not include the practice of relaying shellfish to clean estuarine waters for long periods to promote self cleansing. Depuration is referred to by some authors as purification (Ayres, 1991). Shellfish depuration has been reviewed by Richards (1988) and Ayres (1991), the latter review being undertaken specifically for the New South Wales (NSW) oyster industry.

The process of transferring shellfish to clean waters to allow natural cleansing prior to consumption is not a recently developed concept. Such a practice was widespread in many Mediterranean countries last century (Canzonier, 1988). In the early 1900s, attempts were made to depurate shellfish in land based plants in the United States of America (USA) (Canzonier, 1991). In 1922 William Wells, an engineer employed by the US Public Health Service (USPHS), developed and documented the operation of the first commercial scale depuration plant, located at Inwood, New York State (cited in Canzonier 1991). Early depuration experiments utilised calcium hypochlorite as a seawater disinfection agent. In the USA, depuration technology was initially applied to oysters but later, harvested clams were depurated in land-based tanks. Authors of the 1920's strongly emphasised that the advent of depuration technology was not an excuse for substantial pollution of the raw water supply. At the time of publication, the preferred method of water disinfection in US depuration systems was ultraviolet radiation (UV) (Dressel and Snyder, 1991).

Depuration was originally introduced by authorities in North America in an attempt to reduce outbreaks of typhoid fever associated with the consumption of raw shellfish (Canzonier, 1991). During the 1920's, consumption of contaminated Eastern oysters (*Crassostrea virginica*) resulted in several outbreaks of typhoid fever. Such outbreaks stimulated debate on the limitations of depuration in disease prevention and led to the development of protocols for the analysis of the microbial content of shellfish growing waters as an indicator of sanitary quality. In the USA, microbiological standards were established for shellfish growing waters and a major reliance on growing area classification has subsequently developed (Dressel and Snyder, 1991). The reliability of coliform standards as a single indicator of water quality has been questioned in recent times and this has led to as yet unrewarded, intensive research to identify more reliable indicators of the sanitary quality of shellfish and shellfish growing waters.

Depuration procedures have been adopted in shellfish producing areas throughout the world and within the volume edited by Otwell *et al.* (1991), reports are provided on depuration, quality assurance and sanitation procedures in the USA, Canada, UK, Spain, France, Denmark, Italy, Turkey, South East Asia, Australia and New Zealand.

In Australia, depuration is only practiced routinely in the state of NSW. It is noteworthy that NSW farmers tend to operate their own depuration plants and that the number of licensed plants relative to production is much higher than in most other countries. In 1996-97 approximately 5,300 tonnes of oysters (73,500 bags each holding approximately 100 dozen oysters) were produced in NSW and the number of registered depuration plants during the same period was 218 (NSW SQAP survey data). Many (59%) of the depuration plants that operate in NSW have a capacity of less than 10 bags (1 standard oyster bag contains approximately 1,000 shellfish). By comparison, in 1991 depuration was practiced in only three states in the USA, - Maine (three plants), Massachusetts (one plant) and Florida (two plants) (Dressel and Snyder, 1991). Depuration plants in the US are generally of large capacity, for example the Massachusetts plant is operated by the state and has 9 tanks, each of which can hold 60 bushels or 26 bags of shellfish (2.3 bushels is equivalent to 1 standard bag of oysters) (Dressel and Snyder, 1991).

Ayres (1991) documents the history of depuration in NSW, and notes that initial attempts were made by farmers to establish systems in the 1960's. Depuration was formally introduced in NSW following a food poisoning outbreak in 1978, involving over 2000 clinical cases of viral gastroenteritis which was attributed to the consumption of contaminated shellfish farmed in the Georges River (Linco and Grohmann, 1980; Murphy *et al.*, 1979). Prior to this outbreak there was little information available regarding the sanitary status of NSW estuaries. From 1978 to 1981, estuaries where oysters were farmed in NSW were sampled by regulatory authorities in an attempt to ascertain the levels of faecal contamination. On the basis of these bacteriological findings estuaries were ranked according to risk, however the methodology used in the sampling regime resulted in errors in the ranking of estuaries, with some estuaries being sampled at low frequency and consequently ranked incorrectly (Ayres, 1991). Depuration was initially introduced to estuaries identified as high risk and by 1983 depuration of all shellfish sold in NSW became a statutory requirement, regardless of the sanitary status of the estuary from where the shellfish were harvested. Ayres (1991) provides an excellent technical description and details the diversity of UV - based depuration systems used in NSW.

The mandatory introduction of depuration in NSW in the absence of a coordinated program of bacteriological monitoring of shellfish production areas, has resulted in a degree of complacency both on the part of government and the shellfish industry during the last 15 years. Since the introduction of depuration in 1978 until the implementation of the NSW Shellfish Quality Assurance Program in December 1997, there has been in all but a few instances, little emphasis and no mechanism, to focus on important issues such as the abatement of water pollution in oyster growing areas and the development of efficient pollution early warning systems and notification protocols, to prevent the harvest of potentially contaminated shellfish. Sections of the NSW shellfish industry have continued to reject the need for compulsory depuration, although in the absence of any coordinated monitoring program, have not had sufficient data to support their argument.

## **1.2. Shellfish and Infectious Disease**

Infectious disease associated with the consumption of shellfish, (defined as bivalve molluscs in this review), has been recognised and documented for over a century (summarised by Richards, 1985, 1988). Historically, bacterial diseases such as cholera and typhoid fever, associated with human faecal contamination of the environment were the

chief concern. In the late nineteenth century these diseases occurred in widespread epidemics, primarily associated with contaminated water supplies rather than with shellfish. The severe impact of these diseases on industrialised societies provided the impetus for the evolution of the new science of microbiology to develop methods to detect and identify these bacteria. Foodborne diseases associated with contaminated shellfish have until relatively recently, only considered bacterial pathogens. As a result of this historical focus on faecal borne bacterial disease, the development of shellfish depuration practice and shellfish sanitation methodology in general, has been strongly influenced and guided by the availability of microbiological techniques.

Shellfish also provide a potential vehicle of transmission for a variety of other infectious or otherwise injurious agents. These include natural constituents of the estuarine environment such as potentially toxic algal species, and introduced agents such as human enteric viruses. The development of improved methods for the detection of these agents is required. Such techniques are an essential precursor to the development of sound experimental design for examining the distribution of these agents in the environment, as well as their uptake and excretion by shellfish.

As shellfish sanitation has evolved, so has the reliance on faecal coliforms as general indicators of the sanitary quality of waters and shellfish. However, faecal coliforms interact with both the environment and shellfish in a manner which is significantly different to that of naturally occurring microbes, such as *Vibrio* spp. The relationship between faecal coliforms and enteric viral agents is different again and this is of principal concern in the future development of shellfish sanitation procedures and monitoring programs. To date, the process of depuration has been established as an effective treatment for shellfish moderately contaminated with bacterial agents of faecal origin. This is highlighted by the method by which the depuration process is monitored, ie by the efficacy of removal of faecal coliform bacteria such as *E. coli*. However the efficacy of the depuration process in reducing other infectious agents has not been conclusively quantified and requires further study.

There are several stages during the production cycle where shellfish destined for human consumption are susceptible to contamination with infectious or toxic agents. During this review, the production process will be outlined and the modes of contamination highlighted. The various potentially pathogenic agents and relevant associated issues will then be discussed. Options available for improving the safety of shellfish destined for human consumption and consequently the viability of industry, will be presented. Where information in the literature is insufficient to enable conclusions to be reached, recommendations regarding future areas of research are proposed.

## **Overview of the Problem**

### **1.3. Shellfish-Associated Disease Outbreaks**

In the United Kingdom (UK) between 1981 and 1988 nearly 80 disease outbreaks were attributed to shellfish, exceeding the total number of such outbreaks recorded in the previous 40 years (West, 1991). In the USA during the period 1973–1987, shellfish were identified as the vehicle of transmission in 6% (= 213) of foodborne disease outbreaks (Bean and Griffin, 1990). However, the significance of these figures must be interpreted

with caution as food related disease outbreaks are often sporadic, are likely to be under-reported and are often of unknown aetiology (Bean and Griffin, 1990; Cliver, 1994a; Stelma and McCabe, 1992). Consequently, the true number of clinical cases of disease directly attributable to the consumption of shellfish may be somewhat higher. Contamination of shellfish growing waters and subsequently shellfish with human faecal material, poses the most significant health risk to consumers, most commonly producing symptoms that are consistent with Norwalk virus gastroenteritis (Ahmed, 1992). However, an undefined risk associated with contamination by bacteria and protozoa of animal origin also exists. At this time, viral agents which are endemic in animal populations do not appear to present such a significant risk (Stelma and McCabe, 1992). In addition, the contamination of shellfish with various biotoxins derived from blooms of toxic algal species represents a significant threat to public health. Toxic algae are of particular concern due to the variety of toxins produced, many of which are highly potent and potentially lethal (Hall, 1991; Shumway, 1990).

#### **1.4. Shellfish Sanitation Strategies and Disease Outbreaks**

Various shellfish sanitation strategies have been employed in shellfish growing areas throughout the world. To date these have encompassed a system of classification of shellfish growing areas based broadly on either water test results (USA) (NSSP 1995 a, b) or meat test results (Europe) (EC Directive 91/492), combined with the use of relaying or controlled depuration.

In Australia, the Australian Shellfish Sanitation Program (ASSP) was introduced in 1988 and is modelled on the US National Shellfish Sanitation Program (NSSP). Shellfish growing areas in the majority of Australian states have been classified on the basis of a sanitary survey and the results of an ongoing strategic water sampling program as described in the ASSP. In NSW, shellfish growing areas have not been classified and shellfish sanitation during the last two decades has primarily relied on the use of depuration. Prior to 1978 there appears to be little information regarding the sanitary status of NSW estuaries and almost 20 years later the status of the majority of NSW estuaries where shellfish are farmed remains largely unknown, despite a number of food poisoning outbreaks associated with contaminated shellfish. Without a fundamental change in the policy of the NSW State Government and the provision of adequate resources, it is unlikely that shellfish growing waters in NSW will ever be classified (Ayres, 1991). The absence of a coordinated system of water quality monitoring in NSW was noted in the 1997 Inquiry into the Management of Sewage and Sewage-By-products in the NSW Coastal Zone Draft Report. The NSW Government has attempted to overcome this problem by forming a State Water Monitoring Coordinating Committee which plans to develop and coordinate the implementation of a State water monitoring program (Anon., 1997a).

In December 1997 the NSW Shellfish Quality Assurance Program (NSW SQAP) was implemented. This compulsory program is primarily funded by industry and incorporates a strategy of estuarine water and shellfish monitoring based on a shoreline survey to identify potential pollution sources, in addition to compulsory depuration. The estuary surveys and continuing monitoring program developed by the NSW SQAP provides a coordinated system of data collation and for the first time in the history of the NSW shellfish industry, there is some understanding of the sanitary status of estuaries where shellfish are cultured. However, the resources of the NSW shellfish industry and the NSW SQAP are limited and

without substantial Government support it is unlikely that estuaries will ever be thoroughly surveyed or sufficient data collated, to permit farming areas to be accurately classified.

## USA

Richards (1985) reviewed shellfish-associated enteric virus illness in the USA and cited more than 100 such outbreaks between 1934 and 1985. These outbreaks occurred despite the operation of the NSSP and each instance appeared to be associated with shellfish that had been harvested from approved waters and as such were not required to be depurated (Desenclos, 1991; Koff *et al.*, 1967; Kohn *et al.*, 1995; Portnoy *et al.*, 1975; Richards, 1985; Rippey, 1989). For example Portnoy *et al.* (1975) described an outbreak of hepatitis A attributed to contamination of oysters farmed in an approved shellfish growing area (see 5.1 Classification of Shellfish Growing Waters). At the time of harvesting, the waters and oysters met bacteriological standards. However, investigations revealed that serious pollution had entered from the Mississippi River two to three months previously, water faecal coliform counts had exceeded standards and the area had been closed to shellfish harvesting. As the pollution abated, the waters complied with faecal coliform standards for at least two weeks prior to harvesting. Portnoy *et al.* (1975) concluded that the bacteriological standards were not adequate for describing an area as “approved”, because they did not reliably indicate virological safety.

Guzewich and Morse (1986) described a number of viral gastroenteritis outbreaks that occurred in some of the north eastern states of the USA and Canada during the 1980's. All of these outbreaks appear to have been caused by shellfish that had not been depurated. Guzewich and Morse (1986) identified another factor which may compromise shellfish food safety, the difficulty of tracing shellfish through the processing chain to the consumer. Apparently, tags were often missing and the processing system was overly complex, with shellfish passing through many hands. Other problems identified included illegal harvesting, improper classification of waterways, inadequate monitoring of water quality and opening of conditional beds too soon after sewage contamination (Guzewich and Morse, 1986).

## Australia

The Australian shellfish industry comprises the large NSW oyster industry which has farmed the native Sydney rock oyster (*Saccostrea commercialis*) for over a century and Pacific oyster (*Crassostrea gigas*) farming enterprises which were developed in the 1980's in Tasmania and South Australia and more recently in the NSW estuary of Port Stephens. Scallop and mussel farming is also conducted in Australia, however on a smaller scale compared to the culture of oysters (O'Sullivan and Kiley, 1996).

The Tasmanian and South Australian shellfish industries have attempted to develop shellfish farms in low population areas or remote locations and consequently, these shellfish farms are generally less vulnerable to the impacts of pollution. These new industries have implemented the ASSP with the support of respective State Governments and to date there have been no major recorded outbreaks of disease associated with the contamination of shellfish product by human effluent.

Historically, the NSW oyster industry also operated in waterways which suffered little impact from human activity. Population pressure has increased dramatically over the last 100 years and today the NSW coastline is the most densely populated area of Australia. Oyster farming is conducted along the length of the NSW coastline, from the Tweed Heads estuary in the north of the State to Wonboyn Lake in the south (see Figure 1). NSW shellfish sanitation has mainly consisted of depuration which has been practiced in NSW since widespread outbreaks of gastroenteritis were associated with oysters harvested from the Georges River in 1978 (Linco and Grohmann, 1980; Murphy *et al.*, 1979). The contamination of the oysters was the result of heavy rainfall that was presumed to have caused sewage overflows into the river (Murphy *et al.*, 1979).

Since the introduction of depuration in NSW several shellfish-related food poisoning outbreaks have been recorded, including further outbreaks of Norwalk virus gastroenteritis associated with Sydney rock oysters harvested from the Georges River in 1990 (Bird and Kraa, 1992). The 1990 outbreak was associated with untreated sewage flowing into a harvesting area as a result of very high rainfall and inadequate sewage line capacity. Bird and Kraa (1992) described this outbreak and noted that although harvesting was suspended after rainfall as standard industry practice, of the 11 samples collected of opened oysters associated with the outbreak, only one met required bacterial standards (NSW Food Act 1989; maximum levels permitted for standard plate count 500,000/g and 2.3/g for faecal coliforms). The situation was further complicated, as oysters which were tested by growers prior to sale and which met the bacteriological standard as well as untested oysters, were implicated in the transmission of Norwalk virus (Bird and Kraa, 1992). This highlights the problem of complete reliance on the faecal coliform standard and the difficulties in effective monitoring of shellfish production and processing. Bird and Kraa (1992) also note smaller outbreaks of Norwalk gastroenteritis in 1988 and 1989.

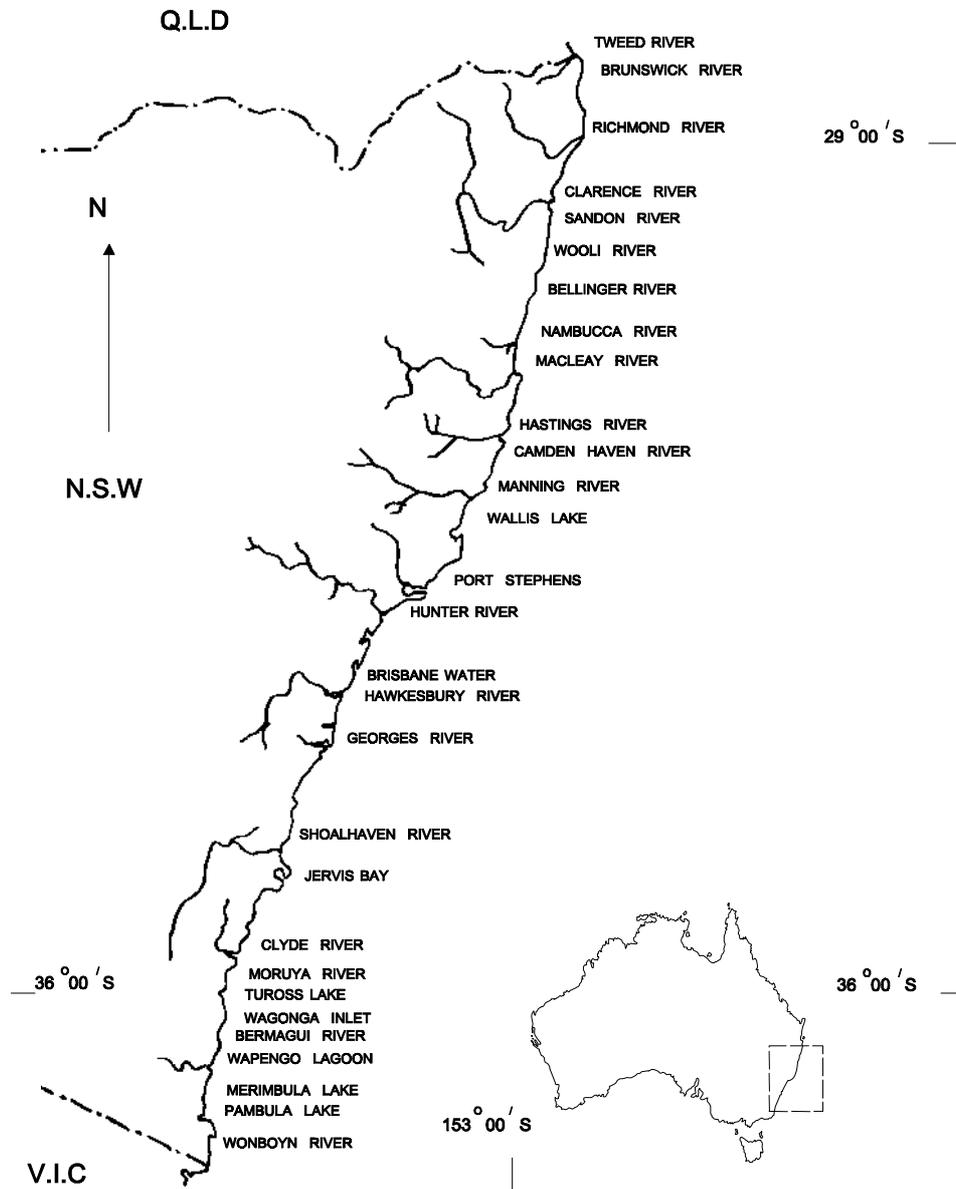
Grohmann *et al.* (1981) studied the incidence of clinical disease in volunteers after consumption of depurated or relayed oysters harvested from two NSW estuaries, Georges River and Brisbane Waters. They demonstrated that some of the volunteers consuming Georges River oysters contracted Norwalk virus gastroenteritis, however no volunteers consuming oysters from Brisbane Waters developed clinical symptoms. They ascribed this difference to the overflow of sewers during heavy rainfall events, in which raw sewage flowed directly into the Georges River, but apparently not into Brisbane Waters. All oysters consumed in the trial had been depurated or relayed and met bacteriological standards.

In September 1996 there was an outbreak of gastroenteritis, (Norwalk virus was suspected as the aetiological agent), which affected more than 160 people. This outbreak was associated with oysters harvested and depurated from Terranora Inlet on the Tweed River (Anon, 1997b). At the time of the outbreak the NSW Shellfish Quality Assurance Program had not been implemented in the Tweed River (Anon, 1997c).

Most recently, an outbreak of hepatitis A was associated with oysters harvested from Wallis Lake (Anon, 1997c). The outbreak occurred in January and February 1997 and 70% of cases were oyster associated with subsequent secondary spread resulting in a total of at least 460 clinical cases (Grohmann, 1997). While it is accepted that sewage contamination of the waterway occurred (Grohmann, 1997), the source of this contamination is not clear. Neither of the sewage systems of the local townships discharge directly into Wallis Lake.

At the time of the outbreak the NSW Shellfish Quality Assurance Program had not been implemented in Wallis Lake.

The NSW SQAP was formally implemented in December 1997. The program is currently restricted to commercially farmed oysters and at this early stage, an appraisal of its impact on shellfish safety is not possible. The program is scheduled for an independent audit after 12 months operation and its success will be largely gauged by the incidence of disease linked to the consumption of oysters produced in NSW.



**Figure 1:** Major NSW oyster producing estuaries.

## 2. Pathogenic Organisms Implicated in Shellfish Associated Disease Outbreaks

### 2.1. Viral Agents

Among the human enteric viruses, Norwalk virus and hepatitis A are currently of chief concern to the NSW oyster industry and other shellfish industries around the world. Outbreaks of gastroenteritis (Norwalk virus) and hepatitis A have been linked to the consumption of shellfish in Australia (Anon 1997a; Bird and Kraa, 1992; Dienstag *et al.*, 1976; Eyles *et al.*, 1981; Murphy *et al.*, 1979) and elsewhere (Koff *et al.*, 1967; Kohn *et al.*, 1995; Morse *et al.*, 1986; Portnoy *et al.*, 1975; Rippey 1989). Klontz and Rippey (1991) reviewed the role of shellfish in the spread of viral disease and noted the common link of sewage contamination of the shellfish prior to harvesting.

Viral agents are potentially the major cause of foodborne illness and the number of outbreaks of virus associated gastroenteritis and hepatitis A due to water/food-borne contamination is increasing (Cliver, 1994a). Cliver (1994b) stated that in all instances of foodborne viral disease the viruses are of human origin; that is, human faecal contamination is a prerequisite of disease. In the case of shellfish, contamination is most likely to occur prior to harvesting (Cliver, 1994b).

Disease outbreaks have occurred after consumption of raw, steamed, incompletely cooked or frozen shellfish (Desenclos, 1991; Dienstag *et al.*, 1976; Koff *et al.*, 1967; Kohn *et al.*, 1995; Linco and Grohmann, 1980; Murphy *et al.*, 1979; Portnoy *et al.*, 1975). Investigation of such epidemics has demonstrated sewage contamination of the shellfish culture areas. Typically, this occurred because large volumes of sewage effluent significantly affected the estuaries in which the shellfish were grown, for example: the Georges River NSW, (Linco and Grohmann, 1980; Murphy *et al.*, 1979) and the Mississippi River USA (Portnoy *et al.*, 1975). However in other cases, instances of localised sewage contamination have resulted in disease outbreaks due to the consumption of shellfish. For example, Kohn *et al.* (1995) attributed an outbreak of Norwalk virus gastroenteritis in Louisiana to the contamination of oysters by infected harvesters. Apparently, crews routinely disposed of their own sewage overboard during harvesting. Prior to acceptance of the current European control program in 1987 (Chris Rodgers, pers. comm. 1998), depurated shellfish were increasingly implicated in enteric viral illness, in countries such as the United Kingdom (Appleton, 1987).

It is apparent that contamination of shellfish with human enteric viral agents presents a significant risk to human health. In addition to hepatitis A and Norwalk viruses, hepatitis E virus is a possible risk in the future as it currently is the most common cause of hepatitis in Asia (Grohmann, 1997).

## 2.2. Bacterial Agents

### 2.2.1 *Vibrio* spp.

As a natural constituent of marine waters, many *Vibrio* spp. may be associated with shellfish. *Vibrio* is a large genus and of the 34 described species, 12 have been identified as being pathogenic or potentially pathogenic to humans (Kelly *et al.*, 1991), the most well known being *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Foodborne outbreaks of disease caused by these bacteria are usually sporadic and involve low numbers of cases (eg. Carnahan *et al.*, 1994; Hansen *et al.*, 1993; Klontz *et al.*, 1993). These cases may (Hansen *et al.*, 1993; Tacket *et al.*, 1984) or may not (Carnahan *et al.*, 1994) be associated with predisposing illness. In *V. vulnificus* infections the most reported type of predisposing condition is liver disease, which is linked to the iron requirements of *V. vulnificus* (Blake *et al.*, 1979; Wright *et al.*, 1993).

Outbreaks of disease caused by *Vibrio* spp. (other than *V. cholerae* 01) are likely to be sporadic and involve individual cases (Blake *et al.*, 1980; Desmarchelier, 1989a). However, high mortality rates have been associated with infection with some *Vibrio* spp. for example a 50% mortality rate with cases of *V. vulnificus* septicaemia (Blake *et al.*, 1979; Kelly *et al.*, 1991; Tacket *et al.*, 1984).

Epidemics of cholera (*V. cholerae* 01) are most commonly associated with contamination of drinking water supplies. However, isolated cases or restricted epidemics of cholera have been attributed to the consumption of contaminated shellfish in the USA and elsewhere in the world (Klontz and Rippey, 1991).

*Vibrio* spp. are typically found in estuarine and marine waters and as such can be expected to be present in shellfish cultured in such waters. Their presence is not correlated with the presence of faecal bacteria, thus obviating the use of faecal coliforms as an indicator. Furthermore, *Vibrio* spp. may increase in numbers in improperly stored shellfish (Son and Fleet, 1980) or during depuration when water temperature is not controlled (Murphree and Tamplin, 1995; Tamplin and Capers, 1992). *V. vulnificus* and *V. parahaemolyticus* constitute ongoing, intermittent risks to shellfish consumers, particularly those who are immunocompromised.

### 2.2.2. Other Infectious Bacterial Agents

Contamination of shellfish by pathogenic bacteria which are not naturally found in estuarine environments may be due to either faecal pollution of shellfish growing areas, direct contamination of shellfish product by infected food handlers, or storage in unhygienic conditions. The number of disease outbreaks due to contamination of shellfish with enteric bacterial agents is generally low compared to other causes of shellfish associated disease. From 1973 to 1987, US Centres for Disease Control (CDC) data indicate that only 13 of 213 shellfish disease outbreaks involved enteric bacterial pathogens including *Clostridium perfringens*, *Salmonella*, and *Shigella* (cited Kvenberg 1991).

There are two main groups of salmonellae strains, general non-host adapted serotypes which cause gastrointestinal upsets and the more serious host-adapted serotypes which are able to invade the host causing septicaemia, for example *S. typhi* and *S. paratyphi* the causative agents of the typhoid fevers (Kvenberg, 1991; Wyatt *et al.*, 1992).

*Shigella spp.* are related to *Escherichia coli* (*E. coli*), have a low infective dose and cause bloody diarrhoea. In 1986 an outbreak of shigellosis in Texas was linked to contaminated oysters harvested from an approved area (Reeve, cited in Klontz and Rippey, 1991). Outbreaks of salmonellosis and shigellosis are not usually associated with shellfish in Australia (Grau, 1989).

Non-pathogenic *E. coli* constitute a normal part of the intestinal flora of humans and animals and these strains are often used as an indicator of faecal pollution. However, there are also three groups of pathogenic strains of *E. coli* which are spread by contaminated water and food and which can cause severe illness and death, especially in young children. Of the pathogenic strains, members of the enterotoxigenic *E. coli* group are becoming more common disease causing agents, for example *E. coli* serotype 0157, which is carried by healthy cattle, causes diarrhoea and occasionally renal failure (Tauxe, 1997; Wyatt *et al.*, 1992).

Other bacterial organisms which are ubiquitous in the environment and may be potentially implicated in shellfish associated disease include *Listeria monocytogenes*, *Clostridium botulinum* the causative agent of botulism, and *Aeromonas hydrophilia* (Klontz and Rippey, 1991; Kvenberg 1991).

### **2.3. Protozoan Parasites**

There is little epidemiological evidence indicating an important role of shellfish in the dissemination of protozoan infections, but *Giardia* sp. and *Cryptosporidium* sp. remain possibilities (Stelma and McCabe, 1992). Fayer *et al.* (1997) demonstrated uptake of *Cryptosporidium parvum* oocysts by *Crassostrea gigas*. The oocysts were found mainly in the gut, but also in haemocytes and retained virulence.

### **2.4. Heavy Metals, Pesticides and Chemical Toxins**

Shellfish have the ability to accumulate heavy metals (Roesijadi, 1996) and lipophilic organic contaminants such as pesticides (Capuzzo, 1996). The rate of accumulation of heavy metals is species specific and depends on the mechanism of absorption and tissue distribution of the agent within the shellfish (Cooper and Langlois, 1982). Elevated levels of chemical contaminants including copper, zinc, tributyl-tin, and PAH and PCB xenobiotics have been identified in oysters, including the Sydney rock oyster and Pacific oyster (Brown *et al.*, 1988; Batley *et al.*, 1989 a,b; Cooper and Langlois, 1982; Nell and Chvojka, 1992; Hardiman and Pearson, 1995; NSW EPA, 1996). Note: the maximum recommended levels provided by the National Health and Medical Research Council are usually adopted as the benchmark for such contaminants. In Tasmania, nausea and vomiting have been reported as a result of consuming oysters farmed in the Derwent estuary approximately 15 km downstream of an Electrolytic Zinc Works (Cooper and Langlois, 1982).

While toxic marine algae are yet to be identified as a major problem in oyster growing areas in NSW, they have caused problems in other states including Victoria, South Australia and Tasmania, where Paralytic Shellfish Poisoning (PSP) is a potential hazard that is managed by monitoring for the dinoflagellate *Gymnodinium catenatum* (Stanley and Brown, 1988, Arnott, 1998). Other algal toxins of concern include; DSP (Diarrhetic Shellfish Poisoning), NSP (Neurotoxic Shellfish Poisoning) and ASP (Amnesic Shellfish Poisoning) (Shumway, 1990; O'Hara, 1993). Shumway (1990) has documented in excess of 100 cases of clinical illness associated with the consumption of marine algal biotoxin contaminated shellfish which have originated from geographical regions other than Australia. In general, problems associated with toxic algal bloom are becoming more severe worldwide (Shumway, 1990).

The effects of biotoxin contamination can range from an unpleasant bitter taste as seen in mussels, scallops and flat oysters (*Ostrea angasi*) in Victoria (Australia) due to the algae *Rhizosolenia chunii* (Parry *et al.*, 1989), to the development of clinical symptoms which may include nausea, memory loss, paresis, paralysis and in some cases death, depending on the toxin involved (Shumway, 1990).

An outbreak may pose very complex problems, as in New Zealand, where up to five species of algae (especially *Gymnodinium breve*, an NSP microalga) were implicated in a major health crisis associated with Greenlip mussels (*Perna canaliculus*) in 1993 (O'Hara, 1993). It should be noted that permissible biotoxin levels vary internationally (O'Hara, 1993).

## **2.5. Summary of Infectious Disease Associated with Shellfish**

Shellfish associated disease outbreaks have occurred around the world despite the operation of quality assurance programs. Depurated and non-depurated shellfish have both been implicated in foodborne disease outbreaks, however it is difficult to assess the significance of many of these outbreaks in terms of failure of sanitation programs or depuration procedures.

It is often difficult to confirm the origin of oysters implicated in outbreaks, epidemiological data is often confidential, and it is often impossible to ascertain if depuration was conducted under optimal conditions, or at all, or whether shellfish were harvested from appropriate areas. Viral agents have been implicated in the largest number of cases and these have generally been associated with large, widespread epidemics. Many of the described outbreaks have been directly attributable to contamination of shellfish growing areas with human faeces, and human enteric viruses appear to be the most common aetiological agent.

Marine biotoxins also contribute significantly to shellfish-associated food safety problems worldwide and the incidence of such outbreaks appears to be increasing.

### 3. Overview of Oyster Physiology

The depuration process exploits the natural physiological functions of the gastrointestinal tract of shellfish. Depuration involves live animals and the success of the process is dependent on the well being of these animals. Understanding depuration requires an appreciation of shellfish physiology, particularly the gastrointestinal tract and feeding activities of the animal. The physiology of oysters will be discussed briefly in the following section to illustrate these processes.

#### 3.1. Gastrointestinal Tract and Feeding Activity

Oysters are filter feeders, with gills providing a surface to trap food particles in addition to performing respiratory functions. This is similar to other bivalves. Microalgae, detritus, zooplankton (minor), free bacteria (inefficiently filtered by oysters because of the typical small size of  $<1.5 \mu\text{m}$ ), attached bacteria (more efficiently consumed because of larger particle size), protozoans, particulate organic matter, and particulate inorganic matter are all potential sources of food for adult oysters. Uptake of dissolved organic matter may also be a significant source of nutrients for larval oysters. Food particles are primarily selected on the basis of size, with preferred items entering the mouth and rejected and unretained particles being directed away to be discarded as pseudofaeces (Newell and Langdon, 1996). Food particles ranging from 3-24  $\mu\text{m}$  in diameter, which is the typical size range of commonly occurring phytoplankton, are appropriate for adult oysters. However, recent unpublished research indicates non selective feeding in relation to larger particle sizes up to 100  $\mu\text{m}$  for Pacific oysters (Nell, 1992; Langdon and Newell, 1996).

Cilia located on the gill surface, transport trapped food particles toward food grooves that deliver mucous-bound strings to the labial palps that surround the mouth. The gut is relatively simple, consisting of an oesophagus, stomach including the gastric shield, style sac, intestine and anus (Langdon and Newell, 1996). The style sac contains the crystalline style, a rotating rod of enzyme/calcareous material which acts to draw the food laden mucus string from the oesophagus (Lester, 1989). The style rotates against the gastric shield, helping to break down algal cells and mix food particles with gastric juices. Fine particles then pass into the tubules of the digestive gland which surrounds the stomach, where intracellular digestion takes place (Lester, 1989). The gut is not highly acidic (examples of pH readings indicate a range of 5.5 - 7.2.). Kueh and Chan (1985) concluded that the bacterial flora in the stomach of Pacific oysters is mainly derived from the external environment but that it may be replaced by a more indigenous flora in the lower gut. *Cristispira* spp. are commonly occurring gut bacteria associated with the style (Langdon and Newell, 1996).

Particle size and abundance are not the only factors influencing feeding activity in oysters. Other important variables include water current (1 cm/second seems optimal for *Crassostrea virginica*), water temperature, salinity, turbidity and dissolved oxygen (Fleet, 1978; Newell and Langdon, 1996). Feeding activity can be affected by both water temperature and temperature change (Shumway, 1996).

Physiological adaptations permit oysters to regulate particle retention efficiency on the gill surface in response to altered concentration of food particles in the environment, whilst concomitantly maintaining respiratory functions. While closely related, pumping rate (velocity of water through the mantle cavity) and filtration rate (volume of water cleared of particles within a particular size range, cleared per unit time) can differ. Clearly, feeding cannot occur without pumping yet pumping activity does not necessarily involve removal of suspended particles (Shumway, 1996).

### **3.2. Environmental Requirements**

The development of spawning broodstock and raising oyster larvae in hatcheries in NSW has necessitated considerable research to identify the optimal environmental requirements of oyster larvae and spat. For example, studies have demonstrated that small Sydney rock oyster spat (1.5 mg) supplied with abundant microalgae, grow best at 30°C in an experimental range of 20-30°C (Nell and Livanos, 1988).

Growout of oysters to market size in NSW is conducted on aquaculture farms located within estuaries and consequently there has not been the impetus to determine optimal environmental requirements for adult oysters.

Satisfactory growth rates have been reported for Pacific oysters cultured in ponds with maximum temperature and salinity values of 34°C and 41‰ (Hughes-Games, 1977). Two unpublished studies by Smith and Nell (a,b year unspecified) indicate that Sydney rock oysters which were acclimatised to winter water temperatures (15°C), filtered effectively at 15°C and 19°C, but not at 11°C in sea water (35‰). Filtration efficiency was determined by the rate of removal of microalgae. Increased water temperature, usually above 20 °C, is recognised as a spawning stimulus for Sydney rock oysters (Frankish, 1989) and Pacific oysters (Arakawa, 1990).

Oysters are able to acclimate to a range of salinities, with Pacific oysters often colonising sites of lower salinity compared to Sydney rock oysters (Holliday and Goard, 1986). Holliday (1995) suggested that 25‰ is the lower end of the optimal range for adults of both species, although there is evidence of tolerance to levels as low as 0-5‰ for adults of both species. It is pertinent to note that a reduction in salinity is also a spawning stimulus for Sydney rock oysters (Frankish, 1989) and Pacific oysters (Arakawa, 1990).

Shumway (1996) reviewed the effects of environmental variables on oyster function with studies concentrating on the Eastern oyster (*Crassostrea virginica*). Temperature and salinity were seen as the major variables, but other factors such as gonad development, light, presence of sperm in the surrounding water, pH, and turbidity could all affect pumping activity or growth. There are significant differences in the results obtained from studies on the effect of silt on oyster physiology. Estimates of sediment levels that have little impact on oyster function vary from < 0.1 g/L to ≥ 0.7 g/L (a density of 0.1 g/L may greatly exceed normal estuarine concentrations) (Shumway, 1996).

Algal removal rate studies indicate that bivalves are generally very tolerant of nitrogenous wastes (ammonia, nitrite and nitrate) (Colt and Armstrong, 1981). However, long term exposure to acidic conditions (pH < 7) can affect growth rates of bivalves (Bamber, 1990).

### 3.3. Summary

Environmental parameters required for optimal feeding and growth of many species of oyster spat have been determined. Similarly, the natural tolerance range of adult oysters to variables such as temperature and salinity is known. Some species differences are evident. Optimal environmental parameters for the function of adult Sydney rock and Pacific oysters cultured in NSW waters have not been determined.

In 1978 Fleet noted that the environmental factors which affect the rate of depuration of the Sydney rock oyster (eg. temperature, salinity and turbidity) were critical control points in the process and required investigation. Similarly, operating parameters which permit effective depuration whilst preventing oysters spawning need to be determined. Despite the commercial practice of depuration in NSW for almost 20 years, many of these questions remain unanswered. The absence of this information has resulted in the adoption of arbitrary environmental parameters for the operation of depuration plants in NSW regardless of their location. These arbitrary standards are incapable of providing an adequate guide to conditions which will promote optimal oyster function to ensure efficient depuration. These issues are further discussed and recommendations are made in sections 7.3 and 7.4.

## 4. Overview of Shellfish Culture and Modes of Contamination

In simple terms, the process of shellfish production as practiced in NSW can be described as:

Growth in an estuary  $\Rightarrow$  optional relay to other estuaries to exploit superior growing conditions  $\Rightarrow$  harvesting  $\Rightarrow$  depuration  $\Rightarrow$  transport  $\Rightarrow$  processing  $\Rightarrow$  consumption.

Contamination of shellfish product can occur at any time in the production chain, however contamination prior to harvesting has been shown to be the most common cause of widespread shellfish associated, food poisoning outbreaks. Depuration for a minimum period of 36 hours is a legal requirement for all shellfish produced in NSW.

### 4.1. Growout

Shellfish are typically cultured in semi-enclosed waterways such as estuaries, as this is the environmental niche in which oysters have evolved and provides shelter and adequate concentrations of food. The growout period is species dependent, with Pacific oysters generally attaining market size in 18 months to 2.5 years and the Sydney rock oyster averaging 3.5 years. Because shellfish filter feed, they have the ability to concentrate particle-bound microbes. If pathogenic agents are present in the culture environment, either as a normal constituent of the environment such as *Vibrio* spp., or introduced for example by the discharge of sewage, shellfish are liable to become contaminated. The risk to human health as a result of such contamination is dependent on a number of variable factors, including the type of pathogenic agent, the level of contamination, the period of time between the contamination event and harvest and the efficacy of post harvest processes such as depuration, in eliminating the contaminant.

### 4.2. Harvesting

Harvested shellfish are subjected to considerable handling. Shellfish harvest involves retrieving farmed shellfish from the estuary, transportation to a land-based facility and subsequent grading, culling and washing. Shellfish may become damaged (breaking of the shell margin with associated loss of mantle fluid) or otherwise stressed (extended emersion during hot weather).

Unpublished work by Smith and Nell (a, year unspecified) compared filtering rates of oysters that had been removed from an estuary, held out of water for two days, and which had been handled gently to those that had been culled in the normal manner. In this study culling was simulated by striking the oysters once with a culling iron, twice shovelling them into a bucket and tipping them onto the concrete floor. It was found that culled oysters demonstrated significantly lower filtration rates during the first four hours of depuration compared to oysters which had been handled gently, however there was no significant difference thereafter.

In summary the following problems may be associated with harvesting:

1. Rough handling (ie exposure to stress such as high/low temperature, physical shock, physical or chemical damage) may damage or stress shellfish, reducing their ability to filter efficiently. Reduced filtering will inhibit the efficacy of the depuration process.
2. Insufficient washing of the shell may increase the likelihood of post-harvest contamination, either during depuration or processing.
3. Insufficient washing of harvested shellfish may increase the turbidity of the depuration system and thus reduce the efficacy of the disinfection process (especially if ultraviolet light is used as the disinfection agent).
4. Shellfish handlers who have contracted a disease and handle product whilst infectious may contaminate shellfish.

### 4.3. Depuration

Particles ingested by shellfish, including infectious agents, may be either digested, sequestered into shellfish tissues or excreted in the faeces. Birkbeck and McHenry (1982) demonstrated rapid uptake and digestion of various bacteria such as *E. coli*, *Staphylococcus aureus* and *Pseudomonas* by Blue mussels (*Mytilus edulis*), to such an extent that bacterial numbers in the overlying water declined. During normal metabolism shellfish excrete faeces that contains microbes that were present in the gastrointestinal tract of the animal.

Depuration is a controlled process that relies on the ability of shellfish to purge their gastrointestinal contents by filtering clean seawater. During the depuration process excreted faeces settles to the bottom of the tank and consequently, is separated from the recirculated water.

Depuration is a very effective process for the elimination of faecal bacteria, such as *E. coli* (eg. Souness and Fleet, 1979), but is less effective for naturally occurring *Vibrio* spp. (Eyles and Davey, 1984; Groubert and Oliver, 1994; Rowse and Fleet, 1984; Tamplin and Capers, 1992). There is also some evidence that *Salmonella* spp. are more difficult to remove by depuration than *E. coli* (Nishio *et al.*, 1981; Murphree and Tamplin, 1995; Son and Fleet, 1980). In laboratory experiments, viruses such as poliovirus may also be removed effectively (Metcalf *et al.*, 1979; Seraichekas *et al.*, 1968), although low levels of viruses may persist in shellfish after depuration (Eyles, 1980). Outbreaks of viral hepatitis and gastroenteritis have been attributed to depurated shellfish taken from heavily contaminated waters (U.S. Food and Drug Administration, 1983; Gill *et al.*, 1983). These outbreaks occurred prior to the introduction of the European Union Program in Britain. In addition, experimental depuration studies have shown the prolonged persistence of hepatitis A virus in oysters (Sobsey *et al.*, 1987). The design, operation, regulation and other issues pertinent to the efficacy of depuration systems used in Australia and elsewhere are comprehensively described and/or reviewed by Ayres (1991), Bird (1994), Fleet (1978), Otwell *et al.* (1991), Richards (1988) and Rodrick and Schneider (1991).

Factors which influence the efficacy of depuration in the removal of microbes include:

1. Shellfish must be healthy and not stressed in order to filter properly. If they do not filter effectively, the gastrointestinal tract will not be purged and consequently any infectious agents within the tract will not be eliminated.
2. Environmental conditions which optimise shellfish filtration are species-specific and may vary according to acclimatisation.
3. Individual shellfish species have different capacities to accumulate and eliminate microbes.
4. Microbial contaminants are not eliminated in a uniform manner and may persist for varying lengths of time in shellfish tissues.
5. Individual shellfish in a batch can show considerable variation in the load of infectious agents that they contain.
6. Microbes may be efficiently removed from shellfish, however if the disinfection process does not inactivate them, they can reinfect the shellfish as the water is recirculated.
7. Naturally occurring microbes such as *Vibrio* spp. are not likely to be removed effectively.

#### **4.4. Transport**

Once depuration has been completed, shellfish may be either processed on site or transported to a processing plant prior to marketing. When removed from water the shell is tightly closed which limits the potential for contamination. Shellfish are commonly sold unopened and alive. The survival time of live shellfish held out of water is species dependent, however all have a limited shelf life. Refrigeration, a technique commonly employed to retard bacterial growth and subsequent food spoilage, is not appropriate for some species of shellfish. Prior to processing, the Sydney rock oyster is generally stored at ambient temperature, avoiding temperature extremes, and if refrigerated will die within four days (Souness *et al.*, 1979). It should be noted that typically, the Sydney rock oyster has a longer shelf life as a live animal under appropriate storage conditions compared to the other oyster farmed in NSW, the Pacific oyster. This fact has an important bearing on product handling and the need to avoid post-harvest species mixing. Residual bacteria within shellfish may multiply during this period, for example *V. parahaemolyticus* (Son and Fleet, 1980) and *V. vulnificus* (Cook, 1994). Consequently, storage and transport of shellfish will be a compromise between conditions which are optimal for shellfish survival and those which encourage bacterial growth.

#### **4.5. Processing**

During processing the shellfish are cleaned, opened and stored under refrigeration until sold. Immediately prior to sale it is common for opened oysters to be stored on ice. Cleaning is highly important and any epiphytic organisms attached to the shell of the

mature oyster such as tunicates, may impact on the resultant quality of the product. Clearly, there is also a possibility of contamination by food handlers operating in unhygienic facilities. Furthermore, it is possible that storage of shellfish on ice immediately before sale, will result in some shellfish experiencing warmer temperatures due to the uneven distribution of product on the ice bed and this may permit spoilage microbes to grow.

Tierney *et al.* (1982) demonstrated that polio virus persisted in *C. virginica*, during refrigeration at 5°C for at least 28 days, which was close to the longest that the shellfish were kept prior to consumption. Metcalf and Stiles (1965) demonstrated survival of coxsackievirus B-3 in *C. virginica* held for 28 days at 5°C. In this case it was demonstrated that the coxsackievirus declined in non-gut tissues but was maintained at initial levels in the gut, suggesting that the gut was unable to inactivate the virus.

*V. vulnificus* will survive for one to two weeks in *C. virginica* and in the Pacific oyster, (*C. gigas*), at refrigeration temperatures (Kaysner *et al.*, 1989). Even low numbers of *V. parahaemolyticus* survived for 10 weeks in homogenates of *C. gigas* refrigerated or frozen (Muntada-Garriga *et al.*, 1995). Son and Fleet (1980) reported that in the Sydney rock oyster, (*S. commercialis*), *V. parahaemolyticus* increased from 65 to 1,500 cells per gram of oyster when stored unopened for four days at 20 - 25°C. Thereafter the numbers of *V. parahaemolyticus* declined.

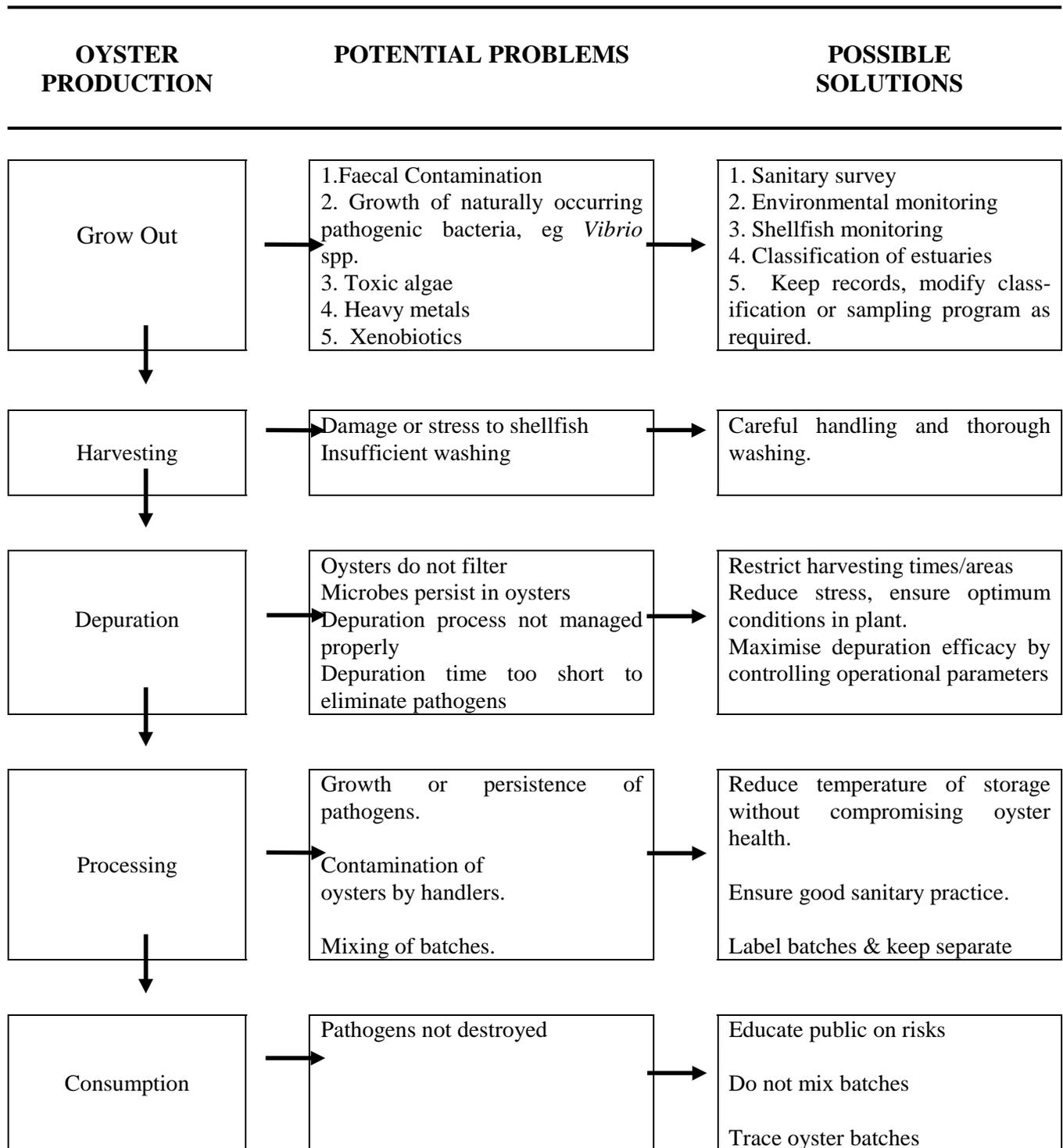
*Salmonella worthington* and *S. agona* persisted for up to 20 days in Blue mussels (*Mytilus edulis*) and oysters (*C. gigas*) stored in air at 10°C (Plusquellec *et al.*, 1994). After heavy contamination ( $7.5 \times 10^4$  cells per 100 g) *S. typhi* persisted, with very little decrease in numbers, in shucked *C. gigas* stored at 10, 5 or -20°C (Nishio *et al.*, 1981).

#### 4.6. Consumption

Shellfish are usually consumed raw and with their gastrointestinal tract intact. Where shellfish are lightly cooked, such as *oysters kilpatrick*, the temperature applied is insufficient to kill many microbes. In an outbreak of Norwalk gastroenteritis investigated by Morse *et al.* (1986), a morbidity rate of 26% was determined for people consuming steamed clams. However, it has been reported that the contaminant *V. vulnificus* can be reduced to undetectable numbers by immersing shellfish in water at 50°C for ten minutes (Cook and Ruple, 1992). These authors claimed that the taste and texture of the oysters was not noticeably affected. As there is no easy method available to the consumer for inactivating infectious agents, it is important that shellfish producers conform to stringent sanitary standards.

#### 4.7. Summary of Factors Affecting the Sanitary Quality of Shellfish

Factors which may affect the sanitary quality of shellfish throughout the production process are summarised in Figure 2. Contamination of shellfish product with pathogenic organisms can occur during any stage of the production process, however faecal pollution of shellfish growing waters is the most common cause of shellfish contamination and subsequent human disease.



**Figure 2:** Summary of Factors Which May Affect the Sanitary Quality of Shellfish.

## 5. Shellfish Sanitation Programs

Various strategies have been employed in shellfish growing areas throughout the world to assure the sanitary quality of shellfish. To date these have either encompassed a system of classification of shellfish growing areas based broadly on water monitoring or shellfish monitoring, often combined with relaying of shellfish to clean waters, or controlled depuration.

### 5.1. Classification of Shellfish Growing Areas

#### USA and Canada

In the USA, the National Shellfish Sanitation Program (NSSP) of the Food and Drug Administration classifies waterways for shellfish harvesting on the basis of a sanitary survey of the growing area, in addition to an ongoing strategic water sampling program. A protocol for depuration has also been established (NSSP, 1990 a, b; NSSP, 1995 a, b). A similar classification system operates in Canada. The NSSP emphasises the importance of the sanitary survey in determining acceptable and unacceptable growing areas and requires that the survey of the waterway be updated annually. The NSSP also establishes contingency plans for marine biotoxins and other deleterious substances (eg pesticides and heavy metals). Shellfish growing waters are then annually classified as:

- *Approved* areas when, under the most unfavourable meteorological, hydrographic, seasonal or point-source conditions, the total coliform median or geometric mean MPN of the water does not exceed 70 per 100 mL and fewer than 10% of the samples exceed a five-tube MPN of 230 per 100 mL (or a three-tube MPN of 330 per 100 mL). In addition, faecal coliforms do not exceed 14 per 100 mL and fewer than 10% of samples exceed a five-tube MPN of 43 per 100 mL (or a three-tube MPN of 49 per 100 mL). At least 15 samples must be analysed. Failure to meet the standards results in temporary closure of the waters.
- *Conditionally approved* areas when the waters are subject to bacterial contamination events, such as from heavy rainfall in the catchment or discharge of sewage. If such an event occurs the State Shellfish Control Agency (SSCA) will conduct a sanitary survey and either approve harvesting if sanitary standards (as above for Approved Waters) are maintained, or close the area until further surveys demonstrate that the sanitary standards have been attained again.
- *Restricted* areas when the waters are subject to limited amounts of pollution such that shellfish must be depurated or relayed prior to sale. Under the most unfavourable meteorological, hydrographic, seasonal or point-source conditions, water samples should not have total coliform levels in excess of 700 per 100 mL with fewer than 10% of samples exceeding 2,300 per 100 mL for a five-tube MPN. In addition, faecal coliforms must not exceed 88 per 100 mL, with fewer than 10% of samples exceeding 260 per 100 mL for a five-tube MPN, or 300 per 100 mL for a three-tube MPN.

- *Conditionally restricted* areas when the waters are subject to intermittent pollution which makes them temporarily unsuitable as a source of shellfish for depuration or relaying. The waters are closed for harvesting until they can meet the sanitary criteria for restricted waters.
- *Prohibited* areas when the level of pollution is such that shellfish are likely to be unfit for human consumption even after depuration or relaying. The harvesting of shellfish is banned from such waters.
- *Unclassified* areas when no sanitary survey has been conducted. Harvesting of shellfish from such areas is banned.

Shellfish harvested from approved or conditionally approved waterways that meet approved area criteria may be harvested and sold directly. Depuration or relay is required for shellfish harvested from conditionally approved areas not meeting approved criteria, and for shellfish harvested from restricted areas or from conditionally restricted areas that meet restricted area classification.

The practice of depuration in the USA is strictly controlled by the SSCA. A scheduled depuration process (SDA) is established for each depuration facility (NSSPb, 1995). This process evaluates the effectiveness of the plant to reduce the number of microorganisms in shellfish harvested from restricted waters on the basis of experimental data. In addition the SDA assesses plant design and construction and process variables such as environmental parameters. This process of verification results in the determination of a maximum initial level of faecal coliforms for each plant. Each batch of shellfish to be depurated must be sampled from the harvest lot and also after the depuration process. All samples are analysed for the presence of faecal coliforms by the MPN method. Rigid sampling regimes specify the number of samples which are required from each batch and the number of samples is dependent on the number of areas harvested and the variability of pollution in each area. End-product standards have been established for each shellfish species commercially harvested. Shellfish are depurated for at least 48 hours.

## Europe

In contrast to the classification system of the United States, European shellfish growing area classification is based on faecal coliform levels in shellfish meat. Annual classifications of growing areas are performed by regulatory agencies in each country. The European Council directive 91/492/EEC sets the standards for each growing area classification.

- *Class A* areas are approved for harvesting shellfish that can be sold directly to the public, with no purification required. Shellfish harvested from Class A areas must contain < 300 faecal coliforms or < 230 *E. coli* per 100 g of mollusc flesh and intravalvular fluid based on a five-tube three-dilution MPN test or other acceptable method. *Salmonella* must also be absent from 25 g of mollusc flesh. In addition, there must be no positive results for Diarrhetic Shellfish Toxin and the amount of Paralytic Shellfish Toxin must be < 80 micrograms per 100 g of mollusc flesh. Radionuclide levels are also specified.

- *Class B* areas are approved for harvesting, but all shellfish must be purified (by relaying or depuration) or cooked by an approved method prior to sale to the public. Shellfish in Class B areas must have < 6,000 faecal coliforms or < 4,600 *E. coli* per 100 g of mollusc flesh in 90% of samples.
- *Class C* areas are not approved for immediate harvesting. Instead shellfish from these areas must be relayed for a prolonged period (at least two months). This process may also be combined with purification to ensure shellfish meet microbiological end-product standards. Alternatively, shellfish may be harvested and cooked by an approved method prior to sale for human consumption. Shellfish from Class C areas must have < 60,000 faecal coliforms per 100 g of mollusc flesh.
- *Class D* areas are those from which harvesting of shellfish is totally prohibited. Shellfish in these areas have > 60, 000 faecal coliforms per 100 g of mollusc flesh. In addition, areas may be designated as prohibited at the discretion of the state.
- Any of the above classified areas may be subject to closure if routine monitoring indicates that sanitary standards are being exceeded. In addition, the EC Directive specifies criteria that must be met for all aspects of shellfish processing (eg. the treatment of shellfish during harvesting, transport and storage). The level of continued monitoring required to maintain the growing area classifications, varies between countries.
- The French appear to have the most comprehensive monitoring system (Maidment, 1996), possibly because of the size of their shellfish industry. The monitoring programs are described by Joanny *et al.* (1993). RNO (Réseau National d'Observation de la Qualité du Milieu Marin) monitors physical parameters (e.g. temperature, salinity, dissolved oxygen) of the coastal waters. This program only surveys a limited number of sites. On the Atlantic coast two to five surveys are made each year, while on the Mediterranean coast, six to twelve surveys are conducted annually. The REPHY (Réseau de Surveillance du Phytoplankton) monitors toxic algal species and the REMI (Réseau de Surveillance du Microbiologique) monitors faecal contamination of shellfish by determining faecal coliform and *E. coli* levels. REMI monitors a total of 345 sampling stations located around the entire coastline on a monthly basis. In shellfish harvesting areas, monitoring is increased to weekly in the event of pollution occurring (Maidment, 1996). The monitoring program is jointly funded by government and the industry.
- Both the European and North American systems have nationally established standards which are implemented, monitored and regulated at a state or regional level. Regardless of the strategy employed, the limitations of current testing methods, sampling regimes and the use of faecal coliforms as indicators of sewage pollution must be recognised. A system that relies totally on water testing will fail at some time. If water samples are not collected at the appropriate time and location, pollution sources and events may be missed. Waters tend to recover from a contamination event more quickly than shellfish, which accumulate and concentrate contaminants. Consequently water monitoring alone may present a false sense of security in relation to shellfish safety. Although the link between polluted water and contaminated shellfish is inferred, without meat testing the actual contaminant load in the shellfish is

not known. A system that relies totally on meat tests also has disadvantages. Meat tests are conducted on only a small sample of the total harvested product and as a consequence, the likelihood of detecting all abnormalities is significantly reduced. The most significant limitation for both sanitation systems is the reliance on the use of bacteriological tests to indicate whether faecal contamination has occurred.

- After examination of both the European and North American shellfish sanitation programs the essential question that arises is; are pathogenic agents present in the water, in the shellfish, or both? To answer this question, we need to determine reproducible methods for the detection of these agents. These techniques need to be sensitive to detect low numbers, relatively simple to perform and economic to enable frequent monitoring. The next question is what is most appropriate to sample, the water, the shellfish or both. The question can be restated: How do we reliably monitor for pathogens to ensure the sanitary quality of shellfish? The simplicity of this question belies the current difficulties in answering it.

### **Factors Complicating Microbial Analysis of Shellfish and Shellfish Culture Waters**

1. The prevalence of microbes in the environment is highly variable in both time and space.
2. Because the concentration of pathogens is often very low, the techniques must be sensitive.
3. Interfering agents, for example tannins, are likely to be present in the environment and will hinder the detection of pathogens.
4. The uptake and excretion of pathogens by shellfish is affected by environmental parameters, such as whether or not the pathogens are bound to particles, the species of shellfish and the health status of the shellfish (and thus their filtering rate).
5. The uptake, tissue distribution and excretion of microbes is not consistent.
6. Some detection techniques are very sophisticated and require high-level laboratory facilities and expertise.
7. The availability, sensitivity, specificity and reproducibility of the detection method.

### **5.2. HACCP-Based Shellfish Quality Assurance Programs**

Infectious disease has been associated with the consumption of shellfish produced under the most stringent sanitation programs, including shellfish which have been harvested from approved waters (under the NSSP system) and those which have been depurated. Human enteric viral agents have been implicated in the largest number of cases and these have generally been associated with large, widespread epidemics. Faecal pollution is the major cause of shellfish contamination. As shellfish may become contaminated at a number of stages during the production process it is unlikely that the incidence of infectious disease can be significantly reduced from existing levels unless the systems for growing, harvesting, processing and monitoring shellfish are addressed.

The most appropriate method for ensuring the quality of shellfish and increasing consumer safety is the implementation of a hazard analysis critical control point (HACCP) based quality assurance program. HACCP based programs are currently being introduced into both the European and North American shellfish industries. Similarly the Australia New Zealand Food Authority (ANZFA) is currently drafting regulations which will require the implementation of HACCP based food safety programs for all food businesses in Australia. HACCP is a multistep preventative approach which identifies the potential hazards in each phase of production, sets critical limits for each hazard, develops appropriate controls, monitoring and preventative measures which can be taken if a critical limit is exceeded. Such a system may encompass classifying and monitoring of waterways, monitoring of the product, the establishment of harvest protocols, careful control of the depuration environment, appropriate monitoring throughout the process and standards for storage, labelling and handling of the product.

## 6. Microbes and Biotoxins in the Estuarine Environment

### 6.1. Detection of Microbes and Biotoxins

Methods employed for the detection of microbes involve either culture of the organism, immunological reactions or identification of nucleic acids specific to the target species. Mammalian bioassays are used as a screening test to detect the presence of biotoxins, however identification of specific biotoxins requires the use pharmacological assays, immunoassays or chromatographic analyses such as high performance liquid chromatography (HPLC).

#### 6.1.1. Detection Methods Based on Culture of the Organism

##### 6.1.1.1. Faecal Coliforms Including *Escherichia coli*

Detection of faecal coliform organisms is of primary importance, given that these bacteria are used to indicate faecal contamination of shellfish and waterways. Currently, the preferred methods for enumeration of faecal coliforms, including *E. coli*, are the Most Probable Number (MPN) or the Membrane Filtration (MF) method (Eyles and Davey, 1989). The MPN is useful for both water and shellfish samples, however the MF method is most appropriate for water samples. Grabow *et al.* (1991) found that the MF method yielded significantly lower counts of total and faecal coliforms in shellfish homogenates compared to the MPN method. This is believed to be due to the prefiltering of the homogenate prior to membrane filtration in the MF method, resulting in the loss of a significant number of bacteria. The MPN detection method takes 4 to 7 days and requires replicate samples for maximum accuracy. Appropriate laboratory standards are required to enable comparisons within and between laboratories. Lightfoot *et al.* (1994) suggested the use of duplicate split water samples for internal quality control and also listed theoretical 95% confidence limits for comparing the duplicate results.

Other methods that have been considered for the detection and enumeration of faecal coliforms include: the Anderson Baird-Parker method, the fluorogenic methods (usually relying on the activity of enzymes such as  $\beta$ -glucuronidase) and impedance methods (Eyles and Davey, 1989).

The Anderson Baird-Parker method is an agar plate technique based on the production of indole, allowing the detection of *E. coli*. It is faster than the MPN technique with results being obtained in 24 hours. Although there are questions regarding the sensitivity of this technique (Eyles and Davey, 1989), Yoovidhya and Fleet (1981) were able to detect between 2 and 5 *E. coli* per gram of tissue in Sydney rock oysters. Regulatory authorities specify the use of the most probable number (MPN) technique for bacterial enumeration in shellfish meat, however the speed and sensitivity of the Anderson Baird-Parker technique suggests that it could be useful in an operational sense. For example, this technique is useful in monitoring faecal coliform loads in shellfish prior to harvesting, in order to

determine when contamination loads are too high for effective depuration and it is commonly used for this purpose in NSW.

Fluorogenic methods rely on detecting the presence of bacterial-specific enzymes. While this technique is relatively fast (results in 48 hours), it has been reported to result in high numbers of false positives and lacks specificity (Motes and Peeler, 1991; Van Poucke and Nelis, 1997).

Dupont *et al.* (1994) devised a protocol for impedance analysis of *E. coli* in shellfish meat that could be completed in less than 8 hours. This method demonstrates satisfactory reproducibility of test results and could detect 30 or more *E. coli*/100 g of tissue. However, impedance techniques require expensive equipment.

#### 6.1.1.2. *Vibrio* spp.

Standard methods for the identification of *Vibrio* spp. rely on enrichment in alkaline peptone water followed by growth on selective and differential media such as Thiosulphate Citrate Bile Salt agar TCBS (Desmarchelier, 1989a) or Cellobiose-Polymyxin B-Colistin agar CPC (Sloan *et al.*, 1992). The initial enrichment is useful for recovering stressed cells which are less likely to grow in selective media (Hagan *et al.*, 1994; Kaysner *et al.*, 1989).

Given the low numbers of pathogenic *Vibrio* spp. in the environment, the use of the MPN or TCBS together with an enrichment stage, is the recommended identification procedure, as it enables growth of the majority of pathogenic *Vibrio* spp. (Desmarchelier, 1989a). Direct plating of *Vibrio* spp. has been trialed with some success. Sun and Oliver (1995) and Miceli *et al.* (1993) found that the direct plate method gave a mean recovery of 91% when compared to recovery via alkaline peptone water MPN. This technique provided higher counts of *V. vulnificus* than did the MPN method and took 3 days instead of 5 days to complete. However, this technique is reported to be only moderately selective so that a background flora of *Vibrio* spp. also grew in the culture. Sun and Oliver (1995) developed a direct plating media that was far more selective, and thus provided a rapid screening method for *V. vulnificus*. However the increased selectivity of this method is likely to reduce its sensitivity. More field-testing is required before considering replacement of the established two-stage method with a direct plating method.

Neither MPN or direct plating is capable of differentiating whether or not a strain of *Vibrio* spp. is actually pathogenic. Pathogenicity has been correlated with the presence of a thermostable haemolysin (Desmarchelier, 1989a). Kaysner *et al.* (1994b) found that, in shellfish taken from waters in Washington State, USA, only 31 of 118 *V. parahaemolyticus* isolates were positive for the thermostable haemolysin. As the samples that were positive for haemolysin were also urease positive, Kaysner *et al.* (1994b) concluded that the urease test could be a useful screen for pathogenic *V. parahaemolyticus* strains, prior to confirmation with genetic or immunological tests. The virulence of some strains of *V. vulnificus* has been correlated with an opaque colony morphology, as distinct from translucent colonies (Simpson *et al.*, 1987) (see section 7.7.1. Bacterial Agents).

### 6.1.2. Immunological Detection Methods

Immunological detection methods are based on the enzyme-linked immunosorbant assay (ELISA). An antibody specific for a particular antigen is first raised in a host animal. The antibody is isolated from the serum of the host animal and then used to detect the original antigen in a sample. In its simplest form, namely a direct ELISA, the antigen is adsorbed to wells in a plastic microtitre tray. The antibody is then added to the microtitre tray and allowed to react with the sample. Any reaction that occurs is visualised by an enzyme linked to the antibody. Once the enzyme's substrate is added a colour change occurs. The amount of colour in the microtitre trays can be observed by eye or measured in a spectrophotometer. The specificity of the antibody-antigen interaction ensures that only the target antigen responds, thereby distinguishing it from other antigens present. In the case of microbes, this enables the identification of a given pathogen amongst a mixed flora.

A number of studies have described the use of ELISAs for the detection of human pathogens in shellfish (e.g. *V. vulnificus*: Biosca *et al.*, 1997; Parker and Lewis, 1995 and Tamplin *et al.*, 1991 and *V. parahaemolyticus*: Chen and Chang, 1995). Both polyclonal (Biosca *et al.*, 1997; Chen and Chang, 1995; Parker and Lewis, 1995) and monoclonal (Tamplin *et al.*, 1991) antibodies have been used. Because they react with only one epitope of an antigen, (part of an antigen that elicits an immune response), monoclonal antibodies should have a greater degree of antigen specificity than polyclonal antibodies. ELISAs have also been developed for the detection of specific biotoxins and kits are available commercially (eg PSP: Cembella *et al.*, 1990 and DSP: Hungerford and Wekell 1992; Morton and Tindall, 1996; Park, 1995).

Since an epitope may be shared between different species or even unrelated organisms (Dohra *et al.*, 1994), cross-reactivity would be expected to be greater when using polyclonal antibodies. Both Chen and Chang (1995) and Parker and Lewis (1995) observed some cross-reactivity in ELISAs they designed to detect *V. parahaemolyticus* and the haemolysin of *V. vulnificus*. Typically, ELISAs have a detection limit of  $10^4 - 10^6$  cells per mL (Fleet and Grohmann, 1993). This range is greatly in excess of the likely environmental concentrations of pathogens. Therefore, environmental samples are likely to require an enrichment step prior to the ELISA. This additional step would increase sample processing time.

To overcome problems associated with reduced binding of the antigen to the titre plate in the presence of large numbers of other bacteria, as reported by Biosca *et al.* (1997) and Tamplin *et al.* (1991), a 'sandwich' ELISA test can be performed. In this case, a specific antibody is first coated onto the microtitre tray, then the target antigen is applied to the tray and finally the enzyme-linked detection antibody. This system enhances the specificity of capture of the target antigen (Chen and Chang, 1995; Parker and Lewis, 1995).

In general, the main advantages of the ELISA are its specificity, precision and the speed with which large numbers of samples can be analysed (Crowther, 1995). However, as with the other methods, ELISA tests cannot distinguish between strains of bacteria. The full potential of using ELISA tests for the microbiological monitoring of shellfish is yet to be realised. Standard reproducible protocols need to be developed for field use of these techniques.

An alternative immunologically-based detection method uses fluorescent antibodies. Antibodies against a particular antigen are labelled with a fluorescent epitope and applied to the sample. These labelled antibodies can then be visualised in an epifluorescence microscope. This technique allows the pathogen to be easily seen amongst a large number of other species. Huq *et al.* (1990) detected *V. cholerae* O1 in > 63% of samples obtained from Bangladeshi coastal waters using the fluorescent antibody technique. In comparison the organism was detected in < 1% of samples using standard culture techniques.

### **6.1.3. Genetic Techniques for Identifying Pathogens**

Nucleic acid (NA) or gene probes matched against specific nucleotide sequences are able to detect individual species of organisms with very high specificity. With Polymerase Chain Reaction (PCR) technology the technique has become even more sensitive, as the original DNA is enzymatically amplified  $10^6$  times and rapid detection is possible (Fleet and Grohmann, 1993). PCR is a method which is able to provide great sensitivity as well as specificity (Chung *et al.*, 1996). Essentially, instead of growing a particular species of microbe or labelling a surface antigen, NA probes are specific for a sequence of nucleotides. The NA probe is labelled with either a radioactive compound or with an enzyme that can engender a colour reaction, as in an ELISA test. The actual nucleotide sequence selected for detection determines the specificity of the technique, given that some nucleotide sequences are only found in a particular species or strain of bacteria, whilst others are common to larger taxonomic groups.

NA probes have been used to detect a diverse array of pathogens. With respect to aquatic environments and shellfish, examples of probes include those specific for: cholera toxin currently being used by US FDA, (Koch *et al.*, 1993), *V. cholerae* O1 biotypes (Shangkuan *et al.*, 1995), *V. parahaemolyticus* (Lee *et al.*, 1995), culturable (Wright *et al.*, 1993) and nonculturable *V. vulnificus* (Brauns *et al.*, 1991), *Salmonella* spp. (Jones *et al.*, 1993) and enteropathogenic *E. coli* O157 (Jinneman *et al.*, 1995). A modification of the technique has been applied by Kaysner *et al.* (1994a) to enumerate *V. vulnificus* and *V. parahaemolyticus* by DNA-DNA colony hybridisation. In this method the NA probe identifies colonies on an agar plate and thus counts of the target species in the original sample can be made. Brauns *et al.* (1991) were able to detect both culturable and nonculturable cells of *V. vulnificus* with an NA probe specific for the cytotoxin gene.

### **6.1.4. Detection of Viral Agents**

Generally, numbers of viral agents in the environment are too low for detection with gene probes alone (eg. Le Guyader *et al.*, 1993), electron microscopy or by immunoassay (Metcalf *et al.*, 1995; Romalde *et al.*, 1994). Viral agents cannot be cultured rapidly in artificial media, meaning that the use of a short pre-enrichment stages to increase their numbers in a sample is not possible.

Viral detection methods depend on satisfactory initial recovery of the virus from the sample. The common practice is to homogenise the shellfish sample and then concentrate the viral agent in the liquid phase. After separating the liquid from the solids the viral agent is precipitated by acidification. Thus control of pH, salinity and temperature will enable concentration of viruses from shellfish. However, as viral agents react differently to

these environmental parameters (Idema *et al.*, 1991), there is no universally applicable procedure for their recovery (Speirs *et al.*, 1987). Landry *et al.* (1982) used a 1% Cat-Floc solution with beef extract to enhance recovery of polio virus in an acid precipitation method. These authors achieved recoveries of generally greater than 50%, and were able to detect 1.5 to 2.0 plaque-forming units (PFU) per shellfish.

High sensitivity is required in measuring viral numbers in order to distinguish between a sample that does not contain viral agents and a sample in which the levels of viral agent are simply too low to detect. Antigen-capture provides an alternative means of concentrating viruses from shellfish (Deng *et al.*, 1994). However, Atmar *et al.* (1995) noted that the polyclonal antisera available for Norwalk virus was highly specific and reacted only with closely related strains. This would mean that all antisera would have to be used for each sample as the type of strain of Norwalk virus in the sample would be unknown.

Another method of enhancing the process of viral recovery from shellfish is to remove only those organs likely to contain virus, rather than testing the whole animal (Atmar *et al.*, 1995; Canzonier, 1971). This increases the concentration of virus in the sample analysed. Romalde *et al.* (1994) used an in situ RT-PCR technique to locate hepatitis A virus in different organs of oysters (*C. virginica*). They found that the virus was predominantly located in the stomach and the hepatopancreas.

Following recovery, some viral agents can be identified by growth in tissue culture which is a lengthy process, or by ELISA or PCR. Neither hepatitis A nor Norwalk viruses, the major shellfish-borne viral agents of concern for human health, can be reliably propagated in cell cultures (Speirs *et al.*, 1987). However, there are many reports of gene probe techniques (PCR and reverse transcriptase PCR = RT-PCR) for detecting waterborne viruses such as hepatitis A, Norwalk and rotaviruses (reviewed by Metcalf *et al.*, 1995). Such techniques are rapid, sensitive, specific and can also quantify viral numbers. However, they do not distinguish between infectious and noninfectious viral particles (Metcalf, *et al.*, 1995). Cromeans *et al.* (1997) reported the detection of 8 PFU per gram of oyster tissue with a method that extracted total RNA from oysters and amplified hepatitis A RNA by RT-PCR. This method used commercially available materials and the results were available in two days.

Metcalf *et al.* (1995) reviewed environmental virology and concluded that appropriate methods are available for detecting pathogenic viruses in shellfish and water samples, however there were issues that needed to be resolved prior to their broad-scale implementation for regulatory purposes. They considered that further work was required to determine the sensitivity of the tests, that is the minimum number of virus particles capable of detection. For viruses that are unable to be cultured, it is impossible to determine the absolute sensitivity of gene probe techniques, as these techniques detect nonviable nucleic acids as well as infectious viral particles. Many authors (e.g. Atmar *et al.*, 1995; Le Guyader *et al.*, 1993; Metcalf *et al.*, 1995) have commented on the necessity for strict control of protocols to ensure reproducibility of the techniques in the field. At present there are no standard protocols for the recovery and detection of viral agents in food and water (Grohmann, 1997).

### 6.1.5. Detection of Biotoxins

The distribution and prevalence of toxic algae throughout the world has been reviewed by Kelly and Hallegraef (1992) and Shumway (1990). Toxic algal blooms are dynamic and their frequency, duration and geographic spread are increasing worldwide (Shumway, 1990). Biotxin blooms appear to be related to altered land use patterns resulting in eutrophication of coastal waters combined with water temperature and stratification (Gabric and Bell, 1993). There is evidence that toxic species are being introduced to previously unaffected areas via ships' ballast water (Hallegraef *et al.*, 1989)

The pharmacology and analytical methods for the detection of biotoxins have been comprehensively reviewed by Shumway (1990), Sullivan (1988), Hall (1991) and Hungerford and Wekell (1992). Biotxin detection is complicated as often a mixture of toxins with a variety of structural compositions may be present in any one sample (Hall, 1991; Hungerford and Wekell 1992). Two general classes of biotoxin detection methods are recognised; assays which provide a single result and do not distinguish between different active substances which may be present (eg. mouse bioassay) and analyses which determine specific toxins, such as high performance liquid chromatography (HPLC).

Mammalian bioassays are commonly used to screen for the presence of biotoxins as no prior knowledge of toxin composition is necessary if both lipid and water soluble toxins are extracted from a sample (Hungerford and Wekell, 1992). However, mammalian bioassays are time consuming, results are inherently variable and may be affected by extraneous material, lack sensitivity and specificity, and require the sacrifice of laboratory animals (Locke and Thibalt, 1994; Honkanen *et al.*, 1996; Laycock *et al.*, 1997; Park, 1995; Sullivan, 1988). Currently the mouse bioassay is the most commonly used method to detect contaminated shellfish and remains the regulatory standard for PSP and DSP toxins (Honkanen *et al.*, 1996). The mouse bioassay involves injecting an extract of the suspect shellfish tissue into the intraperitoneal cavity of a 20g mouse and observation for signs of toxicity (Sullivan, 1988).

Inherent problems with mammalian bioassays have prompted the development of alternate bioassays, immunoassays and pharmacological assays for the detection of biotoxin contaminants in shellfish. This process has been complicated by the number of toxins involved in each recognised biotoxin syndrome (eg PSP, DSP) and their variable composition. However, PSP and DSP immunoassay (ELISA) test kits are commercially available and recent work involving receptor site assays using neuroblastoma cells and isolated sodium channels for PSP detection appear promising (Laycock, *et al.*, 1997).

A variety of analytical methods including spectroscopic and chromatographic methods have been described for the detection of specific biotoxins (Hungerford and Wekell, 1992). The use of highly sophisticated spectroscopic methods is usually confined to research laboratories. Of the chromatographic methods, HPLC is most commonly used and protocols have been developed for all 4 recognised biotoxin syndromes.

## 6.2. Microbes in the Environment

Viral agents contain nucleic acid which constitutes the genome, surrounded by a protein coat. In some cases there is an additional lipid layer outside the protein coat. Viruses will only replicate inside a host cell. While the virus is outside a host cell, it cannot metabolise or reproduce. Whether the virus will replicate within a host cell is determined by specific interactions between the virus and the cell. Viruses that replicate within bacterial cells are

known as bacteriophages. The ability of a virus to persist in the environment is based on its ability to maintain its infectivity for the host cell and to maintain the integrity of its nucleic acid so that it can replicate after successfully invading the host cell. Viral agents may be inactivated by damage to their surface coat, thus preventing the required specific interaction with the host cell. Alternatively, viral agents may be inactivated by sustaining damage to their genome, as caused by UV radiation.

Bacteria are single-celled microorganisms containing a nucleus. Unlike viral agents, bacteria do not require a host cell as a prerequisite for replication. Bacteria commonly require a continuous source of nutrition for survival. Several species of bacteria that may be associated with shellfish have the ability to form spores (a resting state) and in this resting state do not require nutrition, eg *Clostridium perfringens*. Bacteria such as the faecal coliforms, are adapted for survival within the mammalian gastrointestinal tract. When exposed to marine and estuarine environments these bacteria are deactivated by factors such as UV radiation and osmotic stress, and consequently have a finite lifespan in the environment. Bacteria such as the *Vibrio* spp. are adapted to survive in the estuarine environment. Such bacteria are likely to be continuously present in the environment, compared to faecal coliforms which are present principally after a pollution event and are deactivated faster than they can reproduce.

### **6.2.1. Faecal Bacteria in the Environment and the Concept of Indicator Organisms**

Humans that ingest food or water contaminated with bacteria such as *Salmonella*, *Shigella*, enteropathogenic *E. coli* and *V. cholerae* are likely to develop illness as a result of bacterial colonisation and/or invasion of the gastrointestinal tract. As a result, food regulatory authorities have established standards for acceptable levels of pathogenic bacteria in food. As these pathogenic bacteria are often only present in low numbers, continuous testing for these pathogens can be expensive and difficult. As *E. coli* is present in faeces and is not a normal constituent of the environment, the presence of *E. coli* (nonpathogenic strains) is used as an indicator of faecal contamination of food and water. The presence of *E. coli* in food or water suggests that pathogenic bacteria may be also present. It is important to note that indicator bacteria only imply the likelihood that enteric bacterial pathogens may be present.

Bacteria commonly used as indicators of faecal contamination include:

1. *Escherichia coli*.
2. Faecal coliforms. A less restrictive test that is quicker to perform and includes intestinal bacteria including *E. coli*.
3. Total coliforms. The least restrictive test that demonstrates the presence of bacteria from the intestine, as well as some related species of bacteria normally found in the environment.

It is pertinent to note that these microorganisms, including *E. coli*, are derived not only from human sources of faecal pollution, but also from wild and domestic animals, including birds (Kator and Rhodes, 1991).

The use of environmental indicators to ensure the sanitary quality of shellfish was reviewed in the monograph edited by Hackney and Pierson (1994). Of the three indicators listed

above, total coliforms provide the least information regarding faecal contamination, given that positive results may be obtained for nonfaecal bacteria. The more restrictive faecal coliform and *E. coli* assays are more closely related to faecal pollution. However, care needs to be taken when interpreting the results of these tests as cells in the environment may be subject to sublethal stresses which will inhibit their culture in media, i.e. organisms may be viable but non-culturable. For example at temperatures below 10°C, physiological stress reduces the growth of *E. coli* on the selective microbiological media used for culturing cells (Kator and Rhodes, 1994). Hence standard methods for measuring *E. coli* in water samples may actually underestimate their true numbers. Laboratory procedures to resuscitate stressed cells in nonselective media prior to standard culture and enumeration techniques have been developed, however these measures may not be completely effective (Kator and Rhodes, 1994).

Even with efficient enumeration of faecal coliforms, there still remain significant problems associated with their use as an indicator of the sanitary quality of shellfish and shellfish growing waters. As faecal coliforms are derived from a variety of sources besides human faecal pollution, the public health significance of their presence remains unclear (Kator and Rhodes, 1991). Elevated levels of faecal coliforms are commonly detected in estuaries where no point sources of human faecal pollution have been identified (Kator and Rhodes, 1991). Chai *et al.* (1994) surveyed aerobic bacteria, coliforms and coliphages in the waters of Chesapeake Bay, USA during the shellfish harvest season in 1989. They reported that water samples generally met the bacteriological standard (< 14 faecal coliforms per 100 mL). However, water samples collected from sample sites adjacent to heavily cultivated cropland exceeded the faecal coliform bacterial standard more frequently than samples collected from sites located adjacent to a large urban area. They concluded that sewage treatment of the urban area was efficiently reducing the coliform load in discharged effluent. The significance of faecal coliform levels exceeding standards from the sample sites adjacent to cropland is unclear.

Identification and enumeration of faecal coliforms may not necessarily represent the sanitary quality of shellfish or shellfish growing waters, as other pathogenic bacteria may be present. Kfir *et al.* (1993) found a poor relationship between the faecal coliform count and the presence or absence of *Salmonella* spp. in sea water, shellfish and sewage effluent. These researchers voiced concerns regarding use of the faecal coliform standard to determine the sanitary quality of shellfish. However, their use of the membrane filtration method of microbiological analysis may have contributed to the low numbers of faecal coliforms detected in the study.

There is no consistent correlation between the presence of faecal coliforms and human enteric viral agents in the environment (see 6.2.3 Viral Agents in the Environment). Jaykus *et al.* (1994) reviewed the limitations of the faecal coliform indicator and was unable to suggest an alternative. Burkhardt *et al.* (1992) and Doré and Lees (1995) have suggested the use of male-specific bacteriophage as a possible indicator of faecal pollution, due to its morphologic similarity to some human enteric viruses and its prevalence in sewage effluent. Male-specific bacteriophage is found in low numbers in human faeces, however occurs in significant numbers in sewage, suggesting bacteriophage lysis of enteric bacteria and subsequent multiplication during the sewage treatment process (Havelaar, *et al.*, 1986). Male-specific bacteriophage is also a common constituent of the faeces of certain farm animals (Havelaar, *et al.*, 1986). Consequently, although male-specific bacteriophage may indicate sewage contamination or perhaps animal contamination, its use as an indicator of

nonpoint source human faecal contamination requires careful assessment (Kator and Rhodes, 1994). Kator and Rhodes (1994) concluded that there was little prospect of identifying a universally applicable indicator of human faecal contamination. Richards (1985) proposed the detection of specific pathogenic agents (e.g. hepatitis A virus) as an alternative to the use of indicators, however Kator and Rhodes (1991) suggest that this approach may not be valid due to the unpredictable occurrence of pathogens, variations in pathogen virulence and the necessity for a minimum infective dose to affect a target population.

The closure of shellfish growing areas based on the detection of pathogens by sensitive detection methods such as nucleic acid techniques, in the absence of information relating to the health risk the presence of nucleic acid represents, may produce an untenable situation for the shellfish industry and regulatory bodies in the future (Kator and Rhodes, 1991). This view is supported in the USA, where the FDA suggests viral detection techniques should be limited to developing sanitary control procedures, the investigation of shellfish-borne disease outbreaks and research studies. Routine monitoring of shellfish or growing waters for viral contamination is not recommended due to the technical complexity, time required, high cost and limitations of the detection methods (NSSP, 1995a). Regardless of regulatory dilemmas, the recent development of sensitive detection methods such as nucleic acid techniques have the potential to alter the management of shellfish sanitation programs in the future.

### **6.2.2. *Vibrio* Species of Bacteria in the Environment**

Potentially pathogenic *Vibrio* spp. have been widely isolated from coastal waters and shellfish in surveys around North America: these include *V. vulnificus*, *V. parahaemolyticus* and *V. cholerae*. Unlike pathogenic faecal bacteria, *Vibrio* spp. are believed to constitute part of the natural microbiota of estuarine waters and shellfish.

Generally, *Vibrio* spp. are found in larger numbers during the warmer summer months (O'Neill *et al.*, 1992) and this may require the closure of harvest areas during these periods. In Washington State USA, several harvest areas were closed for three weeks during the summer of 1997 due to a high number of cases of *V. parahaemolyticus*; 18 cases were confirmed and another 13 suspected (Anon, 1997d, Anon 1997e). During colder months, bacterial numbers may decline or enter into a nonculturable state (Oliver and Bockian, 1995; Oliver *et al.*, 1991, 1995). These bacteria have been found in fish that bottom feed on shellfish and it has been suggested that bacteria may also overwinter in these fish, and that they may in fact assist in bacterial dispersion (DePaola *et al.*, 1994).

Human illness associated with *Vibrio* spp. is believed to be contributed to by poor handling and storage of shellfish in warm environments after harvesting (Desmarchelier, 1989a; Matté *et al.*, 1994).

### 6.2.3. Viral Agents in the Environment

Viral agents have been detected in a wide range of shellfish worldwide. The following reports indicate that there does not appear to be a reliable correlation between the presence of viral agents and faecal bacteria:

- Hepatitis A virus was detected in high numbers in cockles and mussels from the Atlantic coast of France. Sixty three percent of samples were found to contain viral agents conformed to the faecal coliform standard of <330 coliforms/100g tissue (Le Guyader *et al.*, 1993).
- Enteroviruses were detected in oysters from closed sites in Louisiana, USA. The sites had been closed due to raw sewage contamination. No correlation was found between faecal coliforms and the presence of enteroviruses, although there was a correlation with the aerobic plate count (Cole *et al.*, 1986).
- Enteroviruses have been detected in Green mussels from New Zealand, and no correlation was found between faecal coliforms and the presence of viral agents (Lewis *et al.*, 1986).
- Vaughn *et al.* (1979) surveyed enteroviruses in waters and shellfish around Long Island, New York. At one of the sites studied, these authors reported little difference in the viral loads of waters open or closed to shellfish harvesting. Nor was there an obvious correlation between faecal coliform counts and viral load in the water samples. At one site no viruses were isolated despite extremely high faecal coliform counts (e.g. 2,300 to 9,300 per 100 mL). These authors concluded that the high faecal coliform counts were derived from large flocks of water birds present in the area.
- Metcalf and Stiles (1965) attempted to isolate enteroviruses from Eastern oysters (*C. virginica*) contaminated by sewage in estuaries in New Hampshire. They isolated 6 virus isolations from the first 10 oyster pools obtained from sample sites up to 4 miles from the nearest sewer outfall. They concluded that this indicated that viruses survived in the environment and could be widely distributed from an initial source.

Several factors are believed to influence the ability of viral agents to persist in the environment. Association with particulate matter has been found to affect the longevity of viral agents in the environment. Goyal *et al.* (1984) attempted to isolate and measure the presence of viral agents from sewage sludge disposal sites off the eastern seaboard of the USA. Enteroviruses were found mainly in sediments (26 of 213 sediment samples), but also in crabs (*Cancer irroratus*) and in one sample from the overlying water (sample volumes = 400 to 800 L). At one of the sites, Enteroviruses were isolated 17 months after sludge disposal had ceased. They concluded that this indicated long-term survival of enteroviruses when associated with sediments, particularly those which contained organic carbon. In most samples positive for viruses, the faecal coliform count was found to be below the level of detection.

Rao *et al.* (1984) compared the numbers of enteroviruses in 103 samples of compacted or fluffy sediments, suspended solids and water taken from Galveston Bay, Texas, USA.

Suspended solids contained the highest number of positive samples for the viral agent (72% of samples with a range of 4 to 40 PFU per 100 gallons), while fluffy sediment (i.e. loose, uncompacted sediment from the sediment-water interface) contained the highest concentrations of viral agent (39 – 398 PFU per 100 gallons, 47% of samples being positive). In contrast, there were fewer positive samples in compacted sediments (5%, 7 to 10 PFU/kg) and water (14%, 3 to 12 PFU/100 gallons). The findings of these authors indicate that the association of viral agents with solids appears to enhance survival, and that sediments may act as reservoirs for the viral agent. The presence of viral agents in the uncompacted sediments would suggest that turbulence may resuspend the viral agents into the water, permitting filtration of the viral agents by shellfish. These results also indicate that surface sediments may be the most appropriate place to sample for viral agents, given the relatively high numbers of viral agents in this region.

In laboratory experiments, Smith *et al.* (1978) reported that enteroviruses such as echo 1, coxsackievirus B3 and A9 and poliovirus 1, survived for longer periods in sediments than in water (in the order of days to weeks), particularly if sewage was present. Vaughn and Metcalf (1975) found that the number of coxsackievirus B-3 viral agents decreased at a similar rate, both in estuarine waters and oysters. The persistence of this virus was strongly influenced by temperature, with a decrease in numbers of viral agents detected during summer in both the water and shellfish.

The interpretation of these virological studies is dependent upon the sensitivity of the method of detection of the viral agent. Results demonstrating zero levels of the viral agent may be more an indicator of the sensitivity of the detection method and not necessarily a true indicator that viral agents were absent from the sample. Since the advent of PCR techniques the sensitivity of virological techniques has been substantially increased. Consequently, repetition of the above studies may demonstrate increased viral numbers in some samples.

#### **6.2.4. Summary of Microbes in the Environment**

1. Pathogenic human viruses and bacteria have been detected in shellfish cultured for consumption. The concentration of such pathogens in shellfish is variable.
2. Although shellfish filtering clean water may remove faecal bacteria, enteric viral agents and *Vibrio* spp. potentially persist for extended periods in shellfish.
3. Enteric viral agents have been shown to survive for long periods in marine or fresh waters, especially in association with solids or organic carbon such as sewage.
4. The sensitivity of the microbe detection method must be determined in order that a zero level result can be interpreted.
5. Shellfish and noncompacted sediments are the most likely sites where viral agents may be detected.
6. By virtue of the fact that *Vibrio* spp. are natural inhabitants of estuarine and marine waters, it is not surprising that they can readily be isolated from shellfish, waters and

sediments. This applies to the known human pathogens such as *V. vulnificus*, *V. parahaemolyticus* and *V. cholerae*.

7. Currently used bacterial sanitary standards may be ineffectual for both viral agents and *Vibrio* spp.

#### **6.2.5. Microbial Contamination of Shellfish Under Laboratory Conditions**

A great deal of research has been directed at establishing variables which affect the uptake and elimination of viral agents from shellfish. Laboratory studies have identified many factors which influence the relationship between viral agents and shellfish, however laboratory studies must be interpreted with caution as they do not necessarily represent the behaviour of either shellfish or pathogens under natural conditions.

Poliovirus is most commonly used as a model for studying the uptake and depuration of viral agents in shellfish. This is largely due to the fact that poliovirus is an enterovirus, and enteroviruses are associated with the human gastrointestinal tract and have also been associated with outbreaks of disease. Methods for culturing poliovirus are well known and the viral agent is easy to quantify, given its cytopathic effect in tissue culture, i.e. by the number of plaque-forming units formed per mL (PFU/mL). Other studies have used bacteriophages, such as coliphages S-13 and  $\phi$ A1-5a or male-specific (F<sup>+</sup>) bacteriophages of *E. coli*, given that they are similar in size to human enteric viral agents. While the culturing of viral agents is not necessarily the most sensitive manner in which to detect the presence of a given virus, it does demonstrate the viability of the virus. This is not the case when gene probes are used.

Studies of artificial contamination of shellfish have resulted in the following significant findings:

1. Microbe accumulation occurs rapidly (Birkbeck and McHenry, 1982; Doré and Lees, 1995; Fayer *et al.*, 1997; Murphree and Tamplin, 1995; Plusquellec *et al.*, 1994; Scotti *et al.*, 1983).
2. The microbe load in individual oysters is highly variable (Cook and Ellender, 1986; Landry *et al.*, 1982; Liu *et al.*, 1966b; Metcalf *et al.*, 1979; Murphree and Tamplin, 1995; Scotti *et al.*, 1983; Seraichekas *et al.*, 1968; Tierney *et al.*, 1982).
3. Accumulation of viral agents by shellfish is more rapid when the viral agent is associated with suspended material (Bedford *et al.*, 1978; Hoff and Becker, 1969; Landry *et al.*, 1982 and 1983; Metcalf *et al.*, 1979), although both mono-dispersed and particle associated viral agents can be accumulated by shellfish (Canzonier, 1971; Di Giralamo *et al.*, 1975; Hoff and Becker, 1969; Landry *et al.*, 1982; Liu *et al.*, 1966b; Seraichekas *et al.*, 1968).
4. The majority of viral agents are associated with the intestinal tract of shellfish, particularly the hepatopancreas (Di Giralamo *et al.*, 1975; Doré and Lees, 1995; Hay and Scotti, 1986; Liu *et al.*, 1966a; Metcalf *et al.*, 1979; Power and Collins, 1990; Romalde *et al.*, 1994).

5. The minimum concentration of viral agents in the water that have been reported to be bioaccumulated by shellfish is in the order of 0.01 PFU per mL (Landry *et al.*, 1982). This concentration is similar to that found in natural waters (Liu *et al.*, 1966b; Metcalf *et al.*, 1979).
6. Increasing the concentration of microbes in the water, leads to higher microbe concentrations in shellfish (Bedford *et al.*, 1978; Canzonier 1971; Chai *et al.*, 1994; Cook and Ellender, 1986; Di Giralamo *et al.*, 1975 and 1977; Liu *et al.*, 1966a; Metcalf and Stiles, 1965; Seraichekas *et al.*, 1968).
7. The species of shellfish and their physiological condition influences the accumulation of microbes by the shellfish. Factors such as the water temperature, salinity, turbidity and flow rate, along with shellfish stress can alter the filtration rates of shellfish and hence affect the accumulation of microbes (Canzonier 1971; Cook and Ellender, 1986; Hoff and Becker, 1969; Liu *et al.*, 1966b; Metcalf *et al.*, 1979; Metcalf and Stiles 1965; Sobsey and Jaykus, 1991).
8. Human viral agents and pathogenic bacteria can persist within live or refrigerated shellfish for periods greater than the usual storage time between harvesting and consumption (Chang *et al.*, 1971; Cook, 1994; Kaysner *et al.*, 1989; Metcalf and Stiles, 1965; Muntada-Garriga, *et al.*, 1995; Nishio, *et al.*, 1981; Plusquellec *et al.*, 1994; Tierney *et al.*, 1982).
9. Human viral agents will persist in viral tissues, although they appear to not replicate within shellfish tissue (Burkhardt *et al.*, 1992; Chang *et al.*, 1971; Metcalf and Stiles, 1965). There is evidence that some bacteriophages will replicate in shellfish if the host bacteria are present. However, this has occurred at temperatures in excess of those normally encountered in an estuarine environment (Burkhardt *et al.*, 1992).
10. High variability in microbe load has been detected between oysters in a group (Cook and Ellender, 1986). This finding suggests that interpretation of studies using large numbers of pooled shellfish should be interpreted with care. Relatively high levels may be present in a sample but restricted to a small number of shellfish.

#### 6.2.5.1. Critique of Experimental Design of Laboratory Studies

Certain factors associated with the experimental design of studies investigating the uptake of microbes by shellfish and their subsequent depuration can make interpretation of the findings difficult. These include:

1. Many studies do not include replicate samples in the experimental design. Given the demonstrated high level of variability in the uptake of microbes by individual shellfish, this makes the interpretation of the results from a single unreplicated experiment difficult.
2. Some studies do not provide an assessment of the viral recovery rate of the technique they used. This makes it difficult to distinguish whether a negative result implies that no viral agents were detected or that none were present.

3. Extrapolating experimental results obtained in the laboratory in relation to the contamination of shellfish with microbes and the depuration of shellfish to the behaviour of the shellfish under natural conditions, must be performed with care. The high levels and rapid rates of microbe contamination often used experimentally are likely to be quite different from the manner of contamination in the field. The rate and level of shellfish contamination has been shown to affect the ability of the shellfish to remove the microbe during depuration.

## **7. Depuration Research**

Although the theoretical principles that underpin depuration are relatively simple, many variables influence the efficacy of the process. These include the design and operating conditions of depuration equipment, the sediment content of depuration water, the initial shellfish pathogen load, the type of pathogen, the tissue distribution of pathogens, the physical condition of the shellfish, and the species of shellfish undergoing depuration.

### **7.1. Principal Types of Depuration Systems**

Three basic types of depuration systems have been adopted for use by NSW oyster growers, as reviewed by Ayres (1991).

1. Fish box-type systems consist of a number of high density polyethylene containers stacked on top of one another. Water is pumped into the top tray and allowed to percolate down through the layers of boxes. Water is then pumped back up to the upper layer (from the lower layer of boxes or a separate reservoir), via a UV disinfection unit. This system minimises the volume of water required, while simultaneously providing relatively high density depuration.
2. Tray-type systems consist of a number of trays positioned above one another. Water is either pumped directly into each tray or allowed to percolate through the trays from top to bottom.
3. Pool-type systems consist of a large tank constructed with a gradient towards one end. Oyster baskets are placed in the tank either in a single layer, or multiple layers, separated by sheets of corrugated fibreglass. Water is pumped into one end of the tank and gravity drained over a weir at the other end to be recirculated following disinfection. This is the most common system in operation in NSW.

### **7.2. Methods of Water Disinfection**

It is important for effective shellfish depuration that the water disinfection system used is capable of inactivating microbes. Currently used disinfection systems employ ultraviolet radiation (UV; Australia, Denmark, Malaysia, New Zealand, Philippines, Singapore, United Kingdom, United States), ozone (France, Spain, 1 plant in NSW, Australia), chlorine (France, Italy, Spain) and iodophors (Italy, Spain). These disinfection methods are reviewed in Otwell *et al.* (1991).

#### **7.2.1. Ultraviolet Disinfection**

Disinfection of depuration water by UV radiation has the advantage of producing few residuals in the water that may contaminate the shellfish. It is for this reason that the USFDA prefers UV disinfection to chlorine or ozone.

Ultraviolet radiation interacts with dissolved oxygen in water to form hydrogen peroxide which is an oxidising agent. Conceivably, the hydrogen peroxide may have some residual

disinfecting capacity, however the production of free radicals may also stress the shellfish and stimulate spawning.

Ultraviolet radiation at a wavelength of approximately 260 nm neutralises microbes by affecting the nucleic acids that form the DNA of the microbe (Bitton, 1994). Sufficient damage is incurred to the genetic components of the microbe to break molecular bonds to induce or catalyze chemical reactions and so to prevent protein transcription and replication (Legan, 1982).

Ultraviolet radiation may not necessarily induce sufficient damage to render all microbes incapable of further replication, because the dose of UV radiation may be insufficient to counteract the phenomenon of photoreactivation. Photoreactivation exploits the repair mechanisms that bacteria have evolved to combat exposure to natural UV radiation and involves the excision of the UV damaged DNA segment and its replacement by a newly synthesised segment (Mechsner *et al.*, 1991; Bitton, 1994; Lindenaar and Darby 1994). Photoreactivation may occur when UV damaged microbial cells are exposed to visible light or alternatively may occur in the dark (Mechsner *et al.*, 1991; Bitton, 1994; Lindenaar and Darby 1994).

#### 7.2.1.1. Variables Influencing the Efficacy of UV Disinfection

The ability of UV radiation to effectively neutralise bacteria and viruses in a depuration system is dependent upon several variables. These include:

1. the dose of radiation produced by the UV unit;
2. the flow rate of water past the lamp; and
3. the penetration of UV light through the water.

Recommended effective UV radiation dose rates vary. Battegelli *et al.* (cited in Bitton, 1994) suggested that the dose required to achieve a 3 log (99.9%) decrease in numbers of infectious agents such as hepatitis A is in the order of 20 mW s per cm<sup>2</sup>. In 1966, a USA standard for potable water required a minimum of 16 mW s per cm<sup>2</sup> at all points of the disinfection chamber (Herrington, 1991). Pilkington (1995a) noted that the accepted dosage has increased over time and is now 30 mW s per cm<sup>2</sup> for potable waters and may be up to 50–60 mW s per cm<sup>2</sup> for secondary wastewater effluent. These doses provide for a greater safety margin, in recognition of findings that environmental microbe strains may be inherently more resistant to UV disinfection than laboratory cultures (Pilkington, 1995a; Vasconcelos and Lee, 1972). Effective dosage of UV radiation is also dependent on the initial microbe load, higher loads requiring a higher dose of UV radiation (Herrington, 1991).

Penetration of the UV radiation through water is affected by suspended particles, water colour and the concentration of dissolved materials (e.g. iron salts or tannins). Shellfish-derived particulate matter may also contaminate the depuration system during operation, and contribute to water turbidity. Examples of this may include animals that are poorly cleaned prior to depuration and those that spawn during depuration, however the latter usually results in the termination of the depuration process (Rowse and Fleet, 1984). Certain suspended particles, such as clay minerals that primarily scatter (rather than absorb)

UV light are believed to have little effect on the efficacy of UV disinfection (Bitton, 1994; Miocevic *et al.*, 1993).

Reduced penetration of UV radiation through the water is believed to reduce microbe deactivation during depuration (Souness and Fleet, 1991). It should be noted that some early studies report deactivation of viral agents in turbid water and that any decrease in disinfection due to increased turbidity may be countered by adjusting water flow rates (Hill *et al.* 1967, 1969).

#### 7.2.1.2. Efficacy of UV Radiation

Laboratory studies clearly indicate that UV radiation effectively inactivates a range of bacterial and viral agents in water as illustrated in Table 1.

A residual microbiota has been reported to remain in shellfish following depuration (Vasconcelos and Lee, 1972). However, most research indicates that pathogenic bacteria are amongst the more sensitive microbes (Bitton, 1994; Chang *et al.*, 1985; Son and Fleet, 1980). An exception to this are the pathogenic *Vibrio* spp., which may constitute a part of the natural microbiota (Vasconcelos and Lee, 1972).

The efficacy of UV radiation in neutralising agents such as hepatitis A virus and Norwalk virus in depuration systems remains unclear and warrants further investigation. This could be assessed using a model virus such as a bacteriophage of similar genome and size to the enteric viruses (Havelaar *et al.*, 1991) which could be added to the depuration system and monitored via plaque formation on lawn cultures of the host bacterium.

**Table 1.** Inactivation of water-borne microbial agents by UV radiation.

| Agent  | UV Dose                           | Flow Rate<br>litres/min | Turbidity  | %<br>Inactivation | Reference                    |
|--|-----------------------------------|-------------------------|------------|-------------------|------------------------------|
| Poliovirus<br>type 1   | 71.6 $\mu$ W per<br>$\text{cm}^2$ | 5-20                    | 70 mg/L    | 100               | Hill <i>et al.</i> ,<br>1967 |
| Poliovirus<br>type 1   | 83 $\mu$ Ws per<br>$\text{cm}^2$  | 44                      | 42-51 mg/L | > 99%             | Hill <i>et al.</i> ,<br>1969 |
| Poliovirus 1,2,3<br>Echovirus 1,2<br>Coxsackievirus<br>A9 and B1<br>Reovirus 1 | 83 $\mu$ Ws per<br>$\text{cm}^2$  | static                  | N/A        | 99.9%             | Hill <i>et al.</i> ,<br>1970 |
| Poliovirus 1   | 30 mW s per<br>$\text{cm}^2$      | static                  | N/A        | 99.9%             | Chang <i>et al.</i> , 1985   |
| <i>E.coli</i><br><i>Salmonella</i><br><i>typhi</i>                             | 6 mWs per $\text{cm}^2$           | static                  | N/A        | 99.9%             | Chang <i>et al.</i> , 1985   |
| <i>Bacillus subtilis</i><br>spores   | 60 mWs per<br>$\text{cm}^2$       | static                  | N/A        | 99.9%             | Chang <i>et al.</i> , 1985   |

#### 7.2.2. Ozone

Ozone is used commercially in some depuration plants in France, and Spain. Ozone is a powerful chemical oxidant that reacts with organic materials, resulting in deactivation of microbes. For Enteroviruses this involves damage to the RNA (Roy *et al.*, 1981).

Bitton (1994) reviewed the use of ozone to disinfect water and noted that it is more effective than chlorine against human rotaviruses. Peeters *et al.* (1989) found that 1.1 mg/L of ozone inactivated up to  $10^4$  oocysts of *Cryptosporidium parvum* within six minutes.

Ozone must be produced on site and is most commonly generated by an electric current. The costs and maintenance difficulties associated with ozone disinfection units are greater than for UV systems (Herrington, 1991). As with UV radiation, suspended solids and dissolved organic carbon will both interfere with ozone activity (Kaneko, 1989; cited in Bitton, 1994).

Due to the possible generation of mutagens associated with ozone use, the USFDA does not currently allow contact of ozone with shellfish (Bitton, 1994). Furthermore, given that ozone is an oxidising agent it may react with shellfish tissue (particularly those high in lipids) and cause rancidity. For these reasons, ozone must be removed from the water prior to it entering the depuration tanks. Removal can be achieved through controlling the input of ozone, resting the water prior to use, using UV to convert the ozone back to oxygen or removing the ozone with granular activated carbon (Bitton, 1994).

Le Paulouë *et al.* (1991) provides a description of ozone in a French depuration unit at Cote Bleue on the Mediterranean coast. The facility at Cote Bleue treats approximately 550 metric tons of shellfish a year. Treatment is required prior to depuration, due to transient pollution of the intake water. Disinfection is performed in a series of three tanks at a maximum rate of 150 m<sup>3</sup> per h. Water is treated in the first two tanks with 0.4 mg/L of ozone for two and four minutes respectively. The water is then held in the third tank for ten minutes, by which time the residual ozone concentration drops to between 0.1 and 0.2 mg per L. Water from this tank is then used in the depuration plant.

### 7.2.3. Chlorine

Chlorine in the form of gaseous chlorine or sodium hypochlorite is the disinfectant of choice for domestic water supplies around the world (Trojan and Hansen, 1989). Enteric bacteria are generally sensitive to chlorine, enteric viruses less so and protozoan cysts are resistant to chlorine (Bitton, 1994).

Chlorine acts as an oxidant, causing damage to the external microbe surface and nucleic acids. A significant disadvantage in the use of chlorine as a disinfection agent is the secondary production of carcinogenic trihalomethanes. Chloramines can be used as an alternative to chlorine disinfection, as their use has not been associated with the formation of trihalomethanes. However, chloramines require longer contact times with microbes than chlorine for effective disinfection (Pilkington, 1995b).

Elevated chlorine levels in water used in depuration facilities may interfere with shellfish pumping (Blogoslawski, 1991), and also impart tastes to the shellfish that could reduce the marketability of the product (Rodrick and Schneider, 1991). Dechlorination of the water with thiosulphate prevents the inhibition of shellfish pumping by chlorine, but the consequent reduction in dissolved oxygen may also affect shellfish physiology (Blogoslawski, 1991).

Water pH and dissolved seawater salts influence the disinfection potential of chlorine. Sharp *et al.* (1980) reported significantly faster neutralisation of Poliovirus at pH 10 than at pH 6. Increased levels of seawater salts such as KCl and NaCl improved chlorine disinfection of water (Berg *et al.*, 1990, Sharp *et al.*, 1980).

As with UV and ozone disinfection, turbid water may also affect the efficacy of chlorination. Increased levels of turbidity may decrease the levels of chlorine available for disinfection of microbes. This is believed to be primarily due to the dissolved organic carbon associated with particulate matter which chelates chlorine reducing the amount available for disinfection, rather than with turbidity *per se* (LeChevallier *et al.*, 1981).

#### 7.2.4. Iodine

Scientific literature regarding the use of iodine or iodophors for disinfection of water in depuration plants is limited. Molecular iodine reacts with water to form hypiodous acid (HOI). The relative proportion of iodine to HOI is determined by the pH. At neutral pH, the proportions will be approximately equal, as the pH rises, HOI increases (e.g. at pH = 8, some 88 % is HOI). At acidic pH values the situation is reversed, with iodine predominating (Trojan and Hansen, 1989). This is significant, given that iodine is more virucidal at alkaline pH's and more bacteriocidal at acid to neutral pH's (Alvarez and O'Brien, 1982).

Iodine can be used to disinfect water supplies, although the US EPA considers that it should only be used for short-term or emergency purposes, given the health implications regarding high doses of iodine in the body (Trojan and Hansen, 1989).

Iodophors have been used in Italy for some molluscan depuration operations. These use a Ciba-Geigy product, at a concentration of 0.5–2 mg/L, which is reported to cleanse up to 90 tonnes of mussels in six to ten hours (Casagrande, 1978). This depuration time is considerably shorter than is currently practiced in NSW and USA depuration facilities. Residual concentrations of iodine in the shellfish, following the iodine based depuration system employed in Italy, are reported to be between 0.5 and 1.0 mg/kg of shellfish. These levels are not considered to constitute a health risk (Casagrande, 1978). The recommended dietary allowance of iodine is between 0.08 – 0.14 mg/day (Trojan and Hansen, 1989).

Sobsey *et al.* (1991) examined the virucidal capacity of 8 and 16 mg/L of iodine in clean and polluted waters at different values of pH and temperature. They reported rapid (< 2 minutes) inactivation of hepatitis A, Polio 1 and Echo 1 viruses in clean water at 25°C and pH = 9.5. Inactivation took ≤ 10 minutes at pH = 9.5 and a temperature of 5°C. At neutral and acidic pH values inactivation took longer, particularly at low temperatures and low iodine concentrations. The concentrations of iodine used by Sobsey *et al.* (1991) are considerably higher than used in the Italian depuration operation.

As with the other disinfection systems described, water colour and sediment levels are believed to influence the efficacy of iodine at alkaline and neutral pH levels, although less so at acidic pH levels (Sobsey *et al.*, 1991). Recently, a new form of iodine disinfectant has been marketed. In this system the iodine is attached to a resin through which water is passed. Iodine dissolves into the water and acts on the microbes present. This system is used by NASA in spacecraft and as a portable disinfection unit for remote locations.

Overall, there is insufficient information to conclude whether or not iodine would be useful as a disinfectant in depuration systems in NSW. Clearly, there are many unknown factors, including the effect of iodine on oysters and the efficacy of iodine in commercial operations that have variable water quality.

### **7.2.5. Summary of Disinfection**

The primary purpose of disinfection units used in depuration plants is to treat source water. A number of methods are currently used commercially to do this. UV disinfection is a simple process which is non-toxic to shellfish and should be maintained as the disinfection system of choice in NSW depuration plants. However, research could be conducted to identify methods which would enhance the efficacy of UV disinfection. An investigation into the virucidal efficacy of UV disinfection in commercial NSW depuration systems is also warranted. An appropriate bacteriophage could be used as a model virus, and the efficacy of UV radiation assessed in relation to water turbidity, colour and UV absorbency in the field.

The use of multiple barriers in the disinfection process may be considered, where each barrier acts to further reduce the likelihood of water contamination. These barriers may include the use of an additional disinfection agent such as ozone, or the use of filters. Most importantly, assessment of the source water for the plant in terms of its microbial status must be considered in evaluating the performance of a plant.

The potential application of products that have recently become available, such as the iodine resins may also warrant further investigation.

## **7.3. Temperature and Salinity of Depuration Water**

The temperature and salinity of water used in depuration plants have been found to significantly influence shellfish physiological processes, (e.g. filtration rate, gastric emptying, spawning), and consequently the efficacy of depuration in the elimination of microbes (Fleet, 1978; Shumway, 1996) (see 3.0 Oyster Physiology). Several studies have demonstrated that the optimal temperature and salinity of depuration process water are related to the ambient environmental conditions at the harvest site, geographic location of the depuration plant, the season and the condition of the oysters undergoing depuration. For example, uncontrolled temperature increases have been demonstrated to stimulate spawning of Sydney rock oysters (Frankish, 1989) and Pacific oysters (Arakawa, 1990) and may promote a build up of microorganisms in the shellfish or depuration water. Uncontrolled temperature reductions may impede the activity of shellfish and as a consequence, the effectiveness of depuration.

Rowse and Fleet (1984) investigated the removal of *Salmonella charity* and *E. coli* from Sydney rock oysters in a commercial depuration plant, operated as described by Souness *et al.* (1979). The oysters were obtained from the Georges River during summer (22–25°C) and winter (12–19°C). Oysters were then artificially contaminated with either *S. charity* or *E. coli*. Ultraviolet disinfection was used to deactivate bacteria in the recirculating depuration water. The optimal temperature for oysters from the Georges River undergoing depuration in this study was found to be between 18–22°C. Regardless of whether the oysters were harvested at 12 or 25°C, they did not exhibit signs of stress during depuration

between 18 and 22°C. At temperatures of 13–17°C depuration was inconsistent and sometimes incomplete. Between 24–27°C, depuration rates were also reduced and the possibility that oysters would spawn increased markedly. Spawning at higher depuration temperatures has been reported by other investigators (Buisson *et al.*, 1981; de Mesquita *et al.*, 1991) and by many commercial depuration plant operators. Consequently, these investigators recognised that the ability to control water temperature in the depuration tank is of paramount importance.

Unpublished work by Smith and Nell (b, year unspecified) reported that Sydney rock oysters acclimatised between 15 and 16°C in Port Stephens showed no significant difference in their ability to filter algae at either 15 or 19°C. However, at 11°C the filtration rate was significantly reduced.

Bird *et al.* (1990) compared the depuration of naturally contaminated Sydney rock and Pacific oysters in recirculating and flow-through commercial depuration systems using UV disinfection for a period of 36 hours. The bacterial standard of 0.5 *E. coli*/g of oyster tissue was used to indicate effective depuration of the oysters. This was achieved when the flow-through system was operated at 23.5–28.5°C, with a water salinity between 34.2 and 36.0‰ and in the recirculation plant when operated at 23–26°C with a water salinity between 24.3 and 25.6‰. The reduction in *E. coli* counts occurred within the first 18 hours of the 36 hour depuration process. In contrast, the numbers of *V. parahaemolyticus* were found to increase in oysters during the first 18 hours of depuration before reducing to approximately 100 bacteria per gram of tissue at the completion of the process (Bird *et al.*, 1990). An increase in *V. parahaemolyticus* numbers during depuration has been found to occur when water temperature has not been controlled (Jones, *et al.*, 1991; Tamplin and Capers, 1992). These findings have implications for the efficacy of depuration when practiced for less than 36 hours, especially when water temperature is not controlled.

Buisson *et al.* (1981) demonstrated that temperature influenced the removal of faecal coliforms from Pacific oysters. With initial loadings in the range 1,000 to 10,000 faecal coliforms per 100 g of tissue, the oysters were successfully depurated within three days to < 230 faecal coliforms per 100 g of tissue at 17 to 21°C. At 10.2°C only a marginal reduction in bacteria was achieved and little or no reduction was observed at 5°C.

Temperature is also believed to influence the rate of elimination of viral agents during shellfish depuration. Power and Collins (1990) reported that the elimination of coliphage  $\phi$ A1-5a from mussels during depuration is less efficient compared to *E. coli*, and is also strongly influenced by temperature. Canzonier (1971) noted that coliphage S-13 was inactivated in seawater at the same rate as in clams held at the same temperature, and concluded that the coliphage S-13 was temperature inactivated. This may present difficulties in the use of bacteriophages as indicators of human enteric viruses, given that the latter are not inactivated at commonly-used depuration temperatures.

The optimal temperature and salinity requirements for the efficient depuration of oysters have not been conclusively determined for all geographic areas in NSW (see Section 3.3). However, limited scientific information is available and NSW authorities have specified temperature and salinity values for depuration process water in the current *Code of Practice for NSW Oysters (1991)*. Compliance with these guidelines is mandatory by way

of a permit condition, and is enforced by the regulatory authority, NSW Health. In the current Code an arbitrary line drawn at Bermagui River (located on the far south coast of NSW), determines the operational temperature range of depuration plant process water. For estuaries located south of the Bermagui line, depuration plants must operate with water temperature in the range of 14 - 25 °C, whilst estuaries located to the north operate at 18 - 27°C.

The application of these arbitrary temperature requirements has caused considerable consternation within the NSW oyster industry. Commercial experience from estuaries located north of Bermagui indicates that these specified temperature ranges may not be appropriate or flexible enough to prevent oysters spawning during depuration, especially when ambient temperatures are in the vicinity of 15 - 18°C and the oyster gonad is well conditioned and ready to spawn. Spawning during depuration is disastrous as the resultant product is unfit for sale, i.e. a thin watery oyster, and the depuration plant must be thoroughly cleaned to remove all traces of the ova and sperm before it can be used again. Plant operators in estuaries south of the Bermagui line question the validity of operating depuration plants at a temperature above 14°C at a time when ambient temperatures are below this level. In estuaries immediately to the north of the Bermagui line, with similar ambient temperatures, the requirement to use temperatures above 18°C is equally open to question. Nell (1985) reported that substantial growth of *S. commercialis* occurred at 12°C, which demonstrates that the acclimated oysters filter efficiently at this temperature. Oyster farmers operating on the far south coast of NSW report good growth rates throughout most of the ambient water temperature range of 7 - 25 °C (Wheeler, pers.comm 1998). Examination of the filtering rates and depuration efficacy of oysters acclimated to the lower temperatures common on the NSW south coast would be useful for farmers operating in these regions.

The *Code of Practice for NSW Oysters (1991)* specifies that depuration process water must have a salinity of at least 18‰ and process water salinity may be artificially increased by no more than 20% of the value of salinity at the harvest site, by the addition of sea salt. This minimum salinity level is less than a previously recommended level of 21‰ (Ayres, 1981). The appropriateness of this minimum salinity level is questionable as low salinity levels may inhibit oyster activity. Salinities between 15 and 20‰ were observed to cause stress to the animals, consequently reducing filtration rates (Rowse and Fleet, 1984).

The use of unrefined sea salt as the modifying agent limits the extent to which salinity may be modified, as the salt mix is deficient in certain trace elements which comprise salt water and consequently it is considered unsuitable for use as a major component of process water (Wood and Ayres, 1977). Industry reports that increasing the salinity of process water by 10-20% appears to inhibit oyster spawning, especially when the practice is coupled with effective temperature control. The complementary use of these methods may reduce the degree of modification required, if either is used in isolation. The success of this regime appears to be logical, as it counteracts the recognised fresh water spawning stimulus for Sydney rock oysters (Frankish, 1989) and Pacific oysters (Arakawa, 1990).

Successful depuration requires an environment that promotes oyster activity whilst ensuring that spawning is avoided. Temperature and salinity are recognised as important spawning triggers and as such must be controlled carefully in a depuration plant. Commercial depuration has been practiced in NSW for 20 years, however the operational

parameters for these critical control points of the depuration process have not been conclusively determined. Consequently, in the absence of scientifically based operating parameters the successful use of depuration by individual operators has largely been due to a trial and error approach in developing appropriate local guidelines. Conversely some operators have had neither the ability nor desire to determine appropriate local parameters for the efficient operation of their depuration facilities and in the absence of appropriate guidelines, may have compromised the effectiveness of the depuration process.

In NSW the behaviour of oysters during depuration at various temperatures and the relationship between oyster behaviour, ambient water temperatures at harvest, the temperature range to which the oysters have become acclimated and the operating temperature of depuration plants need to be confirmed. Similarly, the practice of increasing the salinity of process water coupled with temperature manipulation and the impact of these practices on depuration needs to be examined. This fundamental information is of paramount importance to permit efficient, successful and cost effective operation of depuration facilities in NSW.

### **Recommendation**

*Appropriate, scientifically valid operating parameters which permit effective depuration whilst preventing or reducing the incidence of oyster spawning, need to be determined for depuration plants in NSW.*

### **7.4. Turbidity**

Turbidity is purely an optical effect, caused by the presence of suspended particles in water (Gippel, 1983). The turbidity value measured is dependent not only on the concentration of particles in the water, but also their composition, shape and size (Gippel, 1995). Commonly, turbidity is measured in nephelometric turbidity units (NTU) (Kirk, 1986). Nephelometric turbidimeters measure the amount of light scattering. Turbidity measurement does not provide an indication of light absorbency by dissolved organic coloured compounds such as tannins. Particulate matter is believed to influence both the uptake and removal of microbes by shellfish.

Excessive levels of suspended particulate matter may influence the efficacy of depuration by:

1. concentrating pathogens, thereby increasing their uptake by shellfish;
2. affecting the filtration rate of shellfish, thereby reducing their ability to effectively purge themselves during depuration;
3. reducing the penetration of UV light through the depuration water.

The majority of studies to date report an increased uptake of viral agents when they are associated with solids or organic compounds (Bedford *et al.*, 1978; Hoff and Becker, 1969; Landry *et al.*, 1982 and 1983; Metcalf *et al.*, 1979; Millard *et al.*, 1987). Suspended solids are believed to provide an avenue for concentrating viral agents prior to uptake and also increase the likelihood of uptake during filtration. However, mono-dispersed viral agents are still accumulated by shellfish (Canzonier 1971; Di Giralamo *et al.*, 1975; Hoff and

Becker, 1969; Landry *et al.*, 1982; Liu *et al.*, 1966b; Seraichekas *et al.*, 1968). This is believed to occur via initial absorption of the viral agent by gill mucus and subsequent ingestion of the mucus by the shellfish.

Other studies have not reported an association between turbidity in coastal waters and microbe concentration in the water or accumulation in shellfish (Hoff and Becker, 1969; Goyal *et al.*, 1977, 1979). In some instances, high levels of suspended solids may in fact reduce microbe uptake by reducing shellfish filtration rates. Hamblet *et al.* (1969) found an 18-fold accumulation of virus in oysters held in water with a turbidity of 16–24 NTU, but only a 5-fold increase in oysters at 54–77 NTU.

Sobsey and Jaykus (1991) reviewed depuration of viruses and commented that turbidity does not appear to affect the rate of elimination of viruses during depuration. Hamblet *et al.* (1969) demonstrated a 99.9% clearance of Poliovirus from *C. virginica* at turbidities up to 80 mg per L. However, it should be noted that in this investigation, animals were infected with the viral agents over a period of only 24 hours, which may be different from the manner in which accumulation could occur under natural conditions in the environment, ie prolonged low level exposure.

Bird *et al.* (1990) demonstrated effective removal of *E. coli* from both *S. commercialis* and *C. gigas* in recirculating and flow-through systems, despite observing significant turbidity during the depuration period. The effects of turbidity on the efficacy of UV disinfection of depuration water is discussed in section 7.2.1.1.

#### **7.4.1. Turbidity Standards**

In 1990 the USA standards required depuration water to be of less than 20 NTU (NSSP, 1990b). The 1995 NSSP standard no longer sets a maximum turbidity level but states that “*Turbidity does not exceed a value which will inhibit normal physiological activity and/or would interfere with the process of water disinfection. The maximum allowable level of turbidity must be established for each plant during process verification studies.*” Rodrick and Schneider (1991) reviewed depuration and commented “... *the source of sea water should be taken from an approved water area where waves, currents or other factors do not cause excessive turbidity*”, thereby acknowledging the variability in the type and load of suspended solids likely to occur between different sites.

The latest edition of the NSW Code of Practice (Bird, 1994) states that “*Turbid or coloured water should not be used in the plant unless it has been filtered, settled or treated to ensure it is of sufficient clarity for effective sterilisation.*” Settling and/or filtration can remove suspended solids. Additional treatment, such as foam fractionation, is required to remove dissolved organic compounds (Huguenin and Colt, 1989; Spotte, 1979). Foam fractionators are relatively easy to construct and are commonly used in recirculating aquaculture systems with high dissolved organic compounds to help maintain water quality.

Turbidity caused by suspended particles can decrease UV transmission, however the critical level at which the impact of turbidity is significant remains unknown. Regulatory authorities have attempted to deal with the impact of turbidity on the depuration process in different ways. In the US, verification procedures have been established for depuration

plants (NSSP, 1995b) and in NSW at the time of writing, a conservative maximum level of turbidity of less than 1 NTU for depuration water has been specified by NSW Health, the regulatory authority. The level has not been established on the basis of scientific evidence. Turbidity does not reliably indicate UV transmission and as such is not an appropriate indicator of water quality for use in the oyster depuration process (Gippel, pers.comm. 1997). Measurement of the UV absorbency of the inflow water may provide a more useful control measure. This would allow the monitoring of the majority of factors that may adversely affect UV disinfection. Waters which possess a UV absorbency at 254 nm > 0.15 ( $\approx$ 70% transmission) are considered to be unsuitable for UV disinfection (Pilkington, 1995a). In-line monitors of UV transmission would be preferable, although their use is not yet feasible (Herrington, 1991; Pilkington, 1995a).

Several key questions remain to be answered regarding the effect of turbidity on the efficacy of depuration. These include:

1. Is turbidity the most appropriate indicator of UV transmission?
2. When and where within the depuration system, would it be best to monitor turbidity?
3. Does turbidity vary during the depuration process, or is it largely dependent on the turbidity of the incoming water?
4. At what level does turbidity reduce microbe deactivation during depuration - what is an appropriate maximum turbidity level?
5. What is the most appropriate method of measuring turbidity?

### **Recommendation**

- *Research should be carried out to determine the level at which suspended particles (that cause turbidity)*
  - *interfere with UV light transmission in depuration plants,*
  - *interfere with shellfish filtration and depuration effectiveness.*

### **7.5. The Effect of Initial Pathogen Load on the Efficacy of Depuration**

Initial shellfish microbe loading has been found to significantly affect the ability of the shellfish to subsequently remove microbes during depuration. In addition, different species of shellfish exhibit physiological and behavioural differences which may influence the effectiveness of depuration. Generally, shellfish containing high initial loads of microbes appear to take longer, or are unable to eliminate the microbes during depuration.

Seraichekas *et al.* (1968) reported that the required time to remove poliovirus from *M. mercenaria* increased from 48 to 72 hours when the initial load increased from 20 PFU/ml to 100-1000 PFU/ml. Metcalf *et al.* (1979) reported that high loads of poliovirus and other enteroviruses (>200 PFU/10 clams) were not removed from *Mya arenaria*, even after 6 days of depuration. Cook and Ellender (1986) found that oysters infected with poliovirus were similarly unable to remove the virus during relaying to clean waters when the initial viral load was high.

In naturally contaminated mussels from the Tyne River in the United Kingdom, de Mesquita *et al.* (1991) reported that an initial load > 5,000 *E. coli* per 100 g of mussel could not be successfully reduced by depuration to meet the bacteriological standard of 230 *E. coli* per 100 g/tissue.

As previously discussed, water temperature affects the efficacy of depuration. Buisson *et al.* (1981) reported that the temperature of depuration water also influenced the maximum microbe load that could effectively be reduced to acceptable levels (<230 faecal coliforms/g of tissue). For example, at 10°C their predicted maximum loading was 1,250 faecal coliforms per 100 g of tissue, but at 20°C it increased to 16,000 faecal coliforms per 100 g of tissue.

Clearly it is important to assess the initial contaminant load of shellfish prior to depuration.

### **Recommendation**

- *Methods should be established to define the maximum allowable level of faecal coliforms and other contaminants that may be present in NSW oysters prior to depuration.*

### **7.6. The Effect of Tissue Distribution of Microbes on Depuration Efficacy**

The distribution of microbes within shellfish tissues has been reported to influence their subsequent elimination during depuration. Canzonier (1971), using coliphage S-13 as a model for enteric viruses, noted that shock loadings of infectious agents may result in the majority of infectious agents being confined to the gastrointestinal tract of the shellfish. Within the digestive tract, viral agents may be associated with food or faecal pellets, with mucus (Di Giralamo *et al.*, 1977; Hay and Scotti, 1986; Liu *et al.*, 1966a) or closely associated with the epithelial cells (Hay and Scotti, 1986; Romalde *et al.*, 1994). Such localisation may aid in the effective removal of the agent during depuration. In contrast, prolonged contamination with low levels of infectious agents may result in decreased removal of the agents by depuration, due to dissemination of the pathogen into tissues other than the gastrointestinal tract.

Di Giralamo *et al.* (1975) using high concentrations of poliovirus ( $1.9 \times 10^4$  PFU per ml) to contaminate oysters, observed >3 log increase in poliovirus concentration in the oysters within 12 hours, with dissemination to the mantle, gills and the remaining tissue outside of the gastrointestinal tract. They reported that, after 120 hours of depuration, substantial numbers of viral agents remained in both the digestive system and other tissues. In contrast, Doré and Lees (1995) attributed the inefficiency of F<sup>+</sup> bacteriophage removal from mussels and oysters to maintenance of viral agents in the digestive tract, given that after depuration the viral agent could not be found in any other shellfish tissue.

### **7.7. Depuration of Different Microbes**

As has already been suggested, different types of microbes are purged from shellfish at different rates.

### 7.7.1. Bacterial Agents

The success of depuration in the removal of bacterial agents from shellfish depends on several factors including the species of shellfish, bacterial species, method of contamination and environmental conditions within the depuration plant. Several studies have established that many species of bacteria are effectively eliminated from shellfish and deactivated during the depuration process, as summarised in Table 2.

Research has clearly demonstrated that high concentrations of *E. coli* in shellfish can be efficiently eliminated during the depuration process (Souness and Fleet 1979; Souness *et al.*, 1979; Son and Fleet, 1980; Eyles and Davey, 1984; Murphree and Tamplin, 1995). Souness and Fleet (1979) found that Sydney rock oysters from the Georges River, removed high concentrations of *E. coli* (110 per gram of oyster tissue) within 48 hours at 20°C with the depuration water recirculating through an UV light at a rate of 3 times per hour. However, the aerobic plate count did not fall below 10<sup>4</sup> cells per gram of oyster, indicating a residual microbiota in the animals and/or the development of an UV resistant bacterial community.

Souness *et al.* (1979) noted that further investigation was required concerning the depuration of different species of bacteria. Son and Fleet (1980) studied the natural contamination of Sydney rock oysters by different species of bacteria and the removal of laboratory-grown cultures of these bacteria from oysters. These bacteria included *Bacillus cereus*, *Clostridium perfringens*, *Vibrio parahaemolyticus*, *E. coli*, *Salmonella typhimurium* and *S. senftenberg*. To examine depuration, they used a laboratory aquarium as described by Souness and Fleet (1979). They found that both naturally and artificially contaminated Sydney rock oysters were cleansed (ie. had < 2.3 *E. coli* per gram oyster) within two days. However, once again the aerobic plate count indicated that there was a residual microbiota in the oysters after two days of depuration. Furthermore, different bacteria were removed at different rates, with high levels of *Salmonella* (10<sup>5</sup> or 2 X 10<sup>3</sup>/g tissue) in particular, requiring extended periods of depuration (more than 3 days). This is of significance, given that the presence of any *Salmonella* spp. is considered to represent an unacceptable health risk.

Nishio *et al.* (1981) also reported that low numbers of *Salmonella typhi* persisted in *C. gigas* after more than 9 days of depuration at 15°C with UV disinfection. These authors applied a high contamination load (10<sup>8</sup> cells per mL of sea water) to the oysters and then depurated the shellfish immediately. They noted an initial rapid reduction in *S. typhi*, which they considered reflected removal by defecation. The persistence of low numbers was reported to reflect the capability of *S. typhi* to attach to the intestinal epithelium and subsequently disseminate to other tissues.

Several authors have reported that the potentially pathogenic *Vibrio* spp. of bacteria are not effectively eliminated by depuration. Eyles and Davey (1984) compared aerobic plate counts of *E. coli*, coliforms, and *V. parahaemolyticus* in 16 paired batches of undepurated and commercially depurated oysters. They concluded that bacteria that are part of the typical estuarine microbiota such as *V. parahaemolyticus*, behaved differently from bacteria that were introduced to the estuary by way of pollution. This was in contrast to Son and Fleet (1980) who found no difference in the depuration rate of *Vibrio* spp. and

faecal bacteria. Eyles and Davey (1984) postulated that this may indicate that different mechanisms may be utilised in the removal of bacteria from shellfish.

Jones *et al.* (1991) surveyed *Vibrio* spp. in water and in oysters (*C. virginica*) commercially depurated from an estuary classified as restricted in New Hampshire, USA. While these authors reported a reduction in the number of faecal coliforms, levels of total *Vibrio* spp. ranged from 230 to > 2,400,000 per 100 g of tissue in undepurated oysters and the numbers did not significantly decline after 48 hours of depuration. On more than one occasion, the number of *Vibrio* spp. bacteria were reported to increase during depuration. Other workers have also found that *V. vulnificus* numbers increased during depuration (Tamplin and Capers, 1992; Murphree and Tamplin, 1995). Jones *et al.* noted that the determining factor affecting the presence of these pathogenic bacteria appeared to be temperature, as they were not detectable in either the water or oysters after October, when the water temperature fell below 13°C.

Groubert and Oliver (1994) tagged a strain of *V. vulnificus* with a genetic marker and found that this artificially introduced bacterium was readily removed during depuration of *C. virginica*, but that the total number of *Vibrio* spp. was not greatly reduced. They concluded that laboratory contamination with *V. vulnificus* did not necessarily occur in the same manner as occurred during natural contamination. In the latter, they hypothesised that *V. vulnificus* becomes part of the normal flora of the oyster and is able to associate more strongly with oyster tissue (Tamplin and Capers, 1992). Other authors have suggested that laboratory-contaminated shellfish may depurate faster than naturally-contaminated shellfish (Eyles and Davey, 1984; Groubert and Oliver, 1994; Richards, 1988; Tamplin and Capers, 1992) although this suggestion has been the subject of some debate (Souness and Fleet, 1991).

The persistence of *V. vulnificus* (and other *Vibrio* spp.) is generally attributed to their presence in the normal microbiota of shellfish. The virulence of some strains of *V. vulnificus* has been linked to resistance to phagocytosis by shellfish haemocytes (Kreger *et al.*, 1981). This antiphagocytic factor has been correlated with an opaque colony morphology, as distinct from translucent colonies (Simpson *et al.*, 1987). The opaque colonies are produced when the strain forms a capsule that reduces cell binding to haemocytes (Harris-Young *et al.*, 1993). Decreasing the temperature appears to reduce the amount of capsule material made by *V. vulnificus*, which may partly explain its decreased occurrence during colder seasons (Harris-Young *et al.*, 1993). The opaque morphotype also has an increased resistance to intracellular phagocytic digestion (Harris-Young *et al.*, 1995). Furthermore, the number of haemocytes lacking digestive granules increases during warmer weather (Harris-Young *et al.*, 1995). Consequently, the persistence of *V. vulnificus* in shellfish may result from a combination of environmental, bacterial and shellfish factors. Murphree and Tamplin (1995) concluded that current USFDA depuration regulations need to be re-evaluated in light of the persistence of *Vibrio* spp. during the depuration process.

In conclusion, for bacteria to be deactivated, they must first be removed from the oyster. Given that faecal bacteria, such as *E. coli*, are primarily associated with food particles, they are likely to be removed relatively easily from the gastrointestinal tract of the shellfish and eliminated in the faeces. Bacteria that are part of the natural flora of the oyster, either in the gastrointestinal tract or other tissues, are less likely to be removed during depuration and are likely to be more resistant to phagocytosis.



**Table 2:** Elimination of various bacterial agents from shellfish during depuration.

| Shellfish species              | Microbial contaminant  | Natural/artificial contamination                 | Depuration system   | Water flow rate & temperature | Depuration time      | Residual flora  | Reference                    |
|--------------------------------|--|--|---|-------------------------------|----------------------|---|------------------------------|
| <i>Saccostrea commercialis</i> | <i>E. coli</i><br>110/gram   | natural - Georges River                          | -UV disinfection<br>-recirculation                            | 3 times vol/hour<br>20° C     | 48 hours             | <i>E. coli</i> < 2.3/g<br>APC *10   | Souness and Fleet 1979       |
| <i>Saccostrea commercialis</i> | <i>E. coli</i><br>up to 1,100/gram   | laboratory                                       | -stacked modular design<br>-UV disinfection<br>-recirculation | 3 times vol/hour<br>21° C     | 48 hours             | <i>E. coli</i> <2.3/g   | Souness <i>et al.</i> , 1979 |
| <i>Saccostrea commercialis</i> | <i>Bacillus cereus</i><br><i>C. perfringens</i><br><i>V.parahaemolyticus</i><br><i>E. coli</i> | compared natural and artificial contamination    | -lab aquaria<br>-UV disinfection<br>-recirculation            | 60 litres/hour<br>18 -22 °C   | 48 hours             | both artificial and natural -<br><i>E. coli</i> <2.3/g  | Son and Fleet 1980           |
| <i>Saccostrea commercialis</i> | <i>Salmonella typhimurium</i><br>10 <sup>5</sup> /g  | compared natural and artificial contamination    | -lab aquaria<br>-UV disinfection<br>-recirculation            | 60 litres/hour<br>18 -22 °C   | more than three days | <i>E. coli</i> <2.3/g   | Son and Fleet 1980           |
| <i>Crassostrea gigas</i>       | <i>Salmonella typhi</i>  | artificial 10 <sup>8</sup> cells per mL seawater | -UV disinfection  | 15 °C                         | more than nine days  | low numbers persisted   | Nishio <i>et al.</i> , 1981  |
| <i>Crassostrea virginica</i>   | <i>V. vulnificus</i>   | natural  | -lab aquaria<br>-UV disinfection<br>- 0.2µm filter            | 25 °C<br>15 °C                | 3 days<br>7 days     | up to 10 <sup>6</sup> /g<br>≈ 10 <sup>1</sup> /g  | Murphree and Tamplin 1995    |
| <i>Crassostrea virginica</i>   | <i>V. cholerae</i> 01<br><i>E. coli</i><br><i>S. tallahassee</i>                               | artificial                                       | -recirculation<br>-UV disinfection<br>-5.0µm filter           | 30L/min<br>15, 19 and 25°C    | 48 hours             | - <i>V.cholerae</i> 01 persisted<br>- <i>S. tallahassee</i> inconsistent<br>- <i>E. coli</i> <2.3/g | Murphree and Tamplin 1995    |

\* Aerobic plate count

### 7.7.2. Viral Agents

To date no studies have been undertaken to establish the efficacy of the depuration process to remove viral agents from the Sydney rock oyster. The use of *E. coli* clearance rates during depuration as a means of predicting viral agent removal is questionable. Eyles (1980) found no correlation between the number of viral agents and *E. coli* in duplicate samples of oysters. However, *E. coli* removal may provide a useful indicator of efficient depuration plant operation.

In a review of the literature pertinent to the elimination of viral agents during depuration, Eyles (1980) concluded that viruses could be removed from shellfish, however an undefined low level of contamination was likely to remain in the shellfish.

Persistence of viral agents after depuration in recirculating systems incorporating UV disinfection has been observed in the following studies.

- Faeces-associated poliovirus persisted in *Mya arenaria* for six days or more when the mean initial loadings were two to three viruses per animal (Metcalf *et al.*, 1979). This initial loading is believed to mirror naturally occurring loadings of the viral agent.
- Cricket Paralysis virus (used as a model for human enteric viruses) persisted in *C. gigas* for more than ten days when very high initial loadings (>10,000 infectious units per gram of tissue) were used (Scotti *et al.*, 1983).
- Coliphage S-13 persisted in *M. mercenaria* for more than three days at 25°C - a temperature that causes inactivation of the coliphage (Canzonier, 1971). At temperatures below the inactivation range, the coliphage persisted for  $\geq 144$  hours. In this case, reduction of the coliphage numbers appeared to occur independently of clam activity.
- F<sup>+</sup> bacteriophages persisted for  $\geq 48$  hours, primarily in the gut, in both *C. gigas* and *M. edulis*, despite successful removal of *E. coli* from the shellfish (Doré and Lees, 1995).
- Coliphages and F-specific bacteriophages persisted for considerable periods in naturally-contaminated *M. edulis* (de Mesquita *et al.*, 1991; Power and Collins, 1989). De Mesquita *et al.* (1991) calculated that the time to remove 90% of the bacteriophages was in the range of 600 to 2,400 hours.
- Hepatitis A virus was not actively eliminated over a period of three to five days in *C. virginica* in experimental depuration studies at a range of temperatures and salinities nor with the inclusion of algae as a food source (Sobsey *et al.*, 1987).

Given that the infective dose for viral agents is often low, residual viruses may present a significant health risk. Fleet (1978) concluded that viral agents are effectively removed from shellfish, primarily as there was little epidemiological data for shellfish-associated gastroenteritis or hepatitis A outbreaks in countries that practice depuration. Given that a large proportion of shellfish-associated gastroenteritis cases are of unknown aetiology (Bean and Griffin, 1990; Cliver 1994a), this assumption appears questionable.

### 7.7.3. Comparative Removal Rates of Viral and Bacterial Agents During Depuration

As previously discussed, bacterial and viral contaminants respond differently to the depuration process. The rate of elimination of model viruses during depuration is slower compared to the rate of removal of *E. coli*.

De Mesquita *et al.* (1991) depurated mussels (*Mytilus edulis*) that had been naturally-contaminated in the River Tyne estuary on the northeast coast of England. They found that, at 10°C and 15°C, but not at 5°C, there was a significant difference in the rates of removal of two bacteriophages compared to three bacterial indicators (*E. coli*, faecal streptococci and *Clostridium* spores). Both bacteriophages were removed more slowly than the bacteria, with T90 values (time taken to remove 90% of the infectious agent) in the range of 60 to 2400 hours compared to <100 hours for all bacteria examined.

Doré and Lees (1995) allowed oysters (*C. gigas*) and mussels (*M. edulis*) to be naturally-contaminated in sewage-polluted coastal waters in Dorset, UK. They then monitored the concentration of *E. coli* and F<sup>+</sup> bacteriophage present in the shellfish during depuration. They found that both were rapidly accumulated, mainly in the digestive gland (>90%) and that although *E. coli* was efficiently removed during depuration, F<sup>+</sup> bacteriophage was not. The length of exposure (1 to 2 weeks or more than 6 months) did not affect the efficiency of depuration for either *E. coli* or F<sup>+</sup> bacteriophage.

Scotti *et al.* (1983) compared the removal of *E. coli* and high levels of Cricket Paralysis Virus (CrPV), which is used as a model for human enteric viruses, from artificially contaminated Pacific oysters. These authors reported that depuration removed *E. coli* rapidly (< 3 days), but that CrPV was only poorly removed, even after 10 days of depuration. This report highlighted the wide variability in viral loads that shellfish can contain. Scotti *et al.* (1983) assayed individual oysters during the depuration experiments and found that some values for initial loads were lower than loads in oysters that had been depurated for 9 days. As with any valid research it is essential that experimental designs incorporate sufficient sample numbers and replication in order to determine significant differences in treatments.

### 7.7.4. Biotoxins

Shellfish species accumulate and eliminate biotoxins at different rates. Blue mussels (*Mytilus edulis*) accumulate and eliminate toxins at a rapid rate (Blogoslawski, 1988; Wohlgeschaffen *et al.*, 1992) and for this reason are commonly used as sentinels in biotoxin monitoring programs in the USA. Conversely PSP toxins (Shumway and Cembella, 1994) and domoic acid (ASP) are eliminated slowly from species such as deep sea Atlantic scallops (*Placopecten magellanicus*). Biotoxins are preferentially sequestered into specific tissues, for example PSP toxins and domoic acid are generally concentrated in the digestive gland, mantle and gonadal tissue of scallops (Fremy *et al.*, 1993, Shumway and Cembella, 1994; Wohlgeschaffen *et al.*, 1992). In comparison, Wohlgeschaffen *et al.*, 1992 demonstrated that less than 10% of domoic acid ingested by mussels is found in the tissues and hypothesised that domoic acid, being a hydrophilic toxin unlike the many other biotoxins, was rapidly lost to solution during feeding.

The general response to a biotoxin bloom is closure of the affected harvest areas, and such closures may last many months and have a severe economic impact. Little work has been conducted on the use of depuration for biotoxin contaminated shellfish and some authors are of the opinion that depuration or relay of contaminated shellfish is impractical due to the associated costs and long toxin retention times demonstrated by many species (Arnott, 1998). However, preliminary studies and the increasing occurrence of biotoxin blooms may promote further investigations into the usefulness of depuration in reducing biotoxin contamination. Blogoslawski (1988) reported that depuration of shellfish in ozonised seawater inactivated PSP toxins. However, the technique was not successful for ingested algal cysts or shellfish which had toxin bound to tissues over a long time period. Wohlgeschaffen *et al*, (1992) demonstrated the successful depuration of mussels contaminated with domoic acid under laboratory conditions and proposed that further research to determine the usefulness of rapid depuration of contaminated shellfish may be useful.

### **7.8. Summary of Depuration Efficacy**

Depuration is a process which exploits the natural physiological mechanisms of shellfish to promote purging of the gastrointestinal tract. Depuration involves live animals and the success of the process is dependent on the well being of these animals. The efficacy of depuration may be defined as the extent to which microbial and other contaminating agents are eliminated from shellfish during the process.

Shellfish are depurated in order to reduce the likelihood of transmitting infectious or other injurious agents to consumers. Available literature indicates that depuration can successfully reduce to low levels the number of bacterial and viral agents in moderately polluted shellfish.

The current status of depuration and the factors which affect the efficacy of the process are summarised below.

- Depuration, under appropriate operating conditions, is capable of removing many bacterial species from shellfish, including faecal coliforms.
- The water temperature, salinity and turbidity all influence the efficacy of depuration. These factors must be optimised to maintain the health status of the shellfish in order to maximise the efficacy of depuration.
- It is likely that the optimal conditions for depuration will vary between shellfish species and within a species which has been acclimatised to different environments.
- The initial pathogen load, length of exposure to the pathogen and pathogen distribution within shellfish tissues will each influence the efficacy of depuration. Generally, the efficacy of depuration is decreased when the initial pathogen load is high.
- Ultraviolet radiation as a means of water disinfection during depuration, is relatively efficient and cost-effective. Further research is required to assess methods to enhance UV disinfection and to investigate alternate methods of disinfection.

- Not all bacterial species are removed from shellfish at the same rate during depuration. It is apparent that bacteria that constitute part of the natural microbiota of the shellfish (e.g. *Vibrio* spp.) are less readily removed than introduced bacteria (eg *E. coli*).
- The ability of depuration to effectively eliminate viral agents from shellfish is uncertain. It is apparent that viral agents are capable of remaining in shellfish after the depuration process, and that viral agents generally take a longer period of time compared to bacteria, to be effectively removed from shellfish. Further research is required in this area.

### **Recommendation**

*Further experimentation needs to be done to ascertain the efficacy of depuration for the elimination of viral agents from Sydney rock oysters and Pacific oysters. These studies should consider:*

- *the nature of the contamination (natural versus artificial, and initial load).*
- *environmental parameters during depuration, particularly temperature and salinity.*
- *the environment to which the oysters had been previously acclimatised.*
- *the tissue distribution of the contaminants before and after depuration.*
- *additional disinfection steps e.g. ozonation or prefiltration of the depuration water, on the efficacy of depuration.*
- *a suitable technique for measuring the activity of oysters.*

## References

- Advisory Committee for the Minister for Health Services, 1992. New South Wales Oyster Quality Assurance Program, 43 pp.
- Ahmed, F. E., 1992. Review: Assessing and managing risk due to consumption of seafood contaminated with microorganisms, parasites, and natural toxins in the US. *International Journal of Food Science and Technology*, 27: 243-260.
- Alvarez, M. E. and O'Brien, R. T., 1982. Mechanisms of inactivation of Poliovirus by chlorine dioxide and iodine. *Applied and Environmental Microbiology*, 44 (5): 1064-1071.
- Anon., 1997a. Public inquiry into the management of sewage and sewage by-products in the NSW coastal zone. Draft report, August 1997, NSW EPA.
- Anon, 1997b. Hepatitis A outbreak traced to consumption of Wallis Lake Oysters. *NSW Public Health Bulletin*, 8 (1-2): 1-3.
- Anon, 1997c. Sewage Systems and Oyster Harvesting. NSW Department of Land and Water Conservation, Urban Water Directorate, 18 pp.
- Anon, 1997d. News Release - Cook oysters thoroughly. WA State Department of Health. Press release, 21 August, 1997.
- Anon, 1997e. News Release - State reopens oyster beds. WA State Department of Health. Press release, 15 September, 1997.
- Appleton, H. 1987. Small round viruses: Classification and role in food-borne infections. In: *Novel Diarrhoea Viruses*. Ciba Foundation Symposium. Wiley, Chichester, U.K., pp. 108-125.
- Arakawa, K.Y., 1990. Natural spat collecting in the Pacific oyster *Crassostrea gigas* (Thunberg.). *Marine Behaviour and Physiology*, 17: 95-128.
- Arnott, G. H., 1998. Toxic marine microalgae: a worldwide problem with major implications for seafood safety. *Advancing Food Safety*, 1 (4): 24-34.
- Atmar, R. L., Neill, F. H., Romalde, J. L., Guyader, F. L., Woodley, C. M., Metcalf, T. G. and Estes, M. K., 1995. Detection of Norwalk virus and hepatitis A virus in shellfish tissues. *Applied and Environmental Microbiology*, 61 (8): 3014-3018.
- Ayres, P. A., 1981. Purifying the Sydney Rock Oyster. Report and Advice from Dr Peter Ayres, Equipment Suppliers, Building and Operating Advice. AOFPA, 24 pp.
- Ayres, P. A., 1991. The status of shellfish depuration in Australia and south-east Asia. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Depuration*, CRC Press, Boca Raton, FL, pp. 287-322.
- Bamber, R. N., 1990. The effects of acidic seawater on three species of lamellibranch mollusc. *Journal of Experimental Marine Biology and Ecology*, 143: 181-191.
- Batley, G. E., Mann, K. J., Brockbank, C. I. and Maltz, A., 1989a. Tributyltin in Sydney Harbour and Georges River waters. *Australian Journal of Marine and Freshwater Research*, 40: 39-48.
- Batley, G. E., Fuhua, C., Brockbank, C. I. and Flegg, K. J., 1989b. Accumulation of tributyltin by the Sydney rock oyster, *Saccostrea commercialis*. *Australian Journal of Marine and Freshwater Research*, 40: 49-54.
- Bean, N. H. and Griffin, P. M., 1990. Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles and trends. *Journal of Food Protection*, 53 (9): 804-817.

- Bedford, A. J., Williams, G. and Bellamy, A. R., 1978. Virus accumulation by the rock oyster *Crassostrea glomerata*. *Applied and Environmental Microbiology*, 35 (6): 1012-1018.
- Berg, G., Sanjaghshaz, H. and Wangwongwatana, S., 1990. KCl potentiation of the virucidal effectiveness of free chlorine at pH 9.0. *Applied and Environmental Microbiology*, 56 (6): 1571-1575.
- Biosca, E., Marco-Noales, E., Amaro, C. and Alcaide, E., 1997. An enzyme-linked immunosorbent assay for detection of *Vibrio vulnificus* biotype 2: development and field studies. *Applied and Environmental Microbiology*, 63 (2): 537-542.
- Bird, P., 1992. Critical Hazard Analysis Rating for Oyster Purification. Public Health Department of NSW, 27 pp.
- Bird, P., 1994. Purification Technology for New South Wales Oysters. Public Health Department of NSW, 61 pp.
- Bird, P. and Kraa, E., 1992. Overview of the 1990 viral gastroenteritis outbreak from oysters. Proceedings of the Second International Conference on Shellfish Depuration, Rennes, France, April 1992, pp. 31-36.
- Bird, P., Arnold, G. and Holliday, J., 1990. Purification of Pacific oysters (*Crassostrea gigas*) and Sydney rock oysters (*Saccostrea commercialis*). NSW Department of Public Health Report, 22 pp.
- Birkbeck, T. H. and McHenry, J. G., 1982. Degradation of bacteria by *Mytilus edulis*. *Marine Biology*, 72: 7-15.
- Bitton, G., 1994. Wastewater Microbiology. Wiley-Liss, New York, 478 pp.
- Blake, P. A., Merson, M. H., Weaver, R. E., Hollis, D. G. and Heublein, P. C., 1979. Disease caused by a marine vibrio. *The New England Journal of Medicine*, 300 (1): 1-5.
- Blake, P. A., Weaver, R. E. and Hollis, D. G., 1980. Diseases of humans (other than cholera) caused by vibrios. *Annual Review of Microbiology*, 34: 341-68.
- Blogoslawski, W., 1988. Ozone depuration of bivalves containing PSP: pitfalls and possibilities. *Journal of Shellfish Research*, 7 (4): 702-705.
- Blogoslawski, W., 1991. Enhancing depuration. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Depuration*, CRC Press, Boca Raton, pp. 145-149.
- Brauns, L. A., Husson, M. C. and Oliver, J. D., 1991. Use of the polymerase chain reaction in detection of culturable and nonculturable *Vibrio vulnificus* cells. *Applied and Environmental Microbiology*, 57 (9): 2651-2655.
- Brown, C. and Russo, D. J., 1979. Ultraviolet light disinfection of shellfish hatchery sea water 1. Elimination of five pathogenic bacteria. *Aquaculture*, 17: 17-28.
- Brown, K. R., McPherson, R. G. and O'Grady, A., 1988. Heavy Metals in Oysters from the Georges River, NSW. In: Proceedings Australian Marine Science Association Jubilee Conference, 1963-1988, pp. 57-61.
- Buisson, D. H., Fletcher, G. C. and Begg, C. W., 1981. Bacterial depuration of the Pacific oyster (*Crassostrea gigas*) in New Zealand. *New Zealand Journal of Science*, 24: 253-262.
- Burkhardt, W., III, Watkins, W. D. and Rippey, S. R., 1992. Survival and replication of male-specific bacteriophages in molluscan shellfish. *Applied and Environmental Microbiology*, 58 (4): 1371-1373.
- Canzonier, W. J., 1971. Accumulation and elimination of coliphage S-13 by the Hard clam, *Mercenaria mercenaria*. *Applied Microbiology*, 21 (6): 1024-1031.

- Canzonier, W. J., 1991. Historical perspective on commercial depuration of shellfish. In: W.S. Otwell, G.E. Rodrick and R.E. Martin (eds). Molluscan Shellfish Depuration, CRC Press, Boca Raton, FL, pp. 7-15.
- Capuzzo, J. M., 1996. The bioaccumulation and biological effects of lipophilic organic contaminants. In: V. S. Kennedy, R. I. E. Newell and A. F. Elbe (eds). The Eastern Oyster *Crassostrea virginica*, Maryland Sea Grant College, College Park, Maryland, pp. 539-557.
- Carnahan, A. M., Harding, J., Watsky, D. and Hansman, S., 1994. Identification of *Vibrio hollisae* associated with severe gastroenteritis after consumption of raw oysters. *Journal of Clinical Microbiology*, 32 (7): 1805-1806.
- Casagrande, F., 1978. Effective purification in aquaculture. *Ceiba Geigy Journal* 1: 22-25.
- Cembella, A., Parent, Y., Jones, D., and Lamoureux, G., 1990. Specificity and Cross-Reactivity of an Absorption-Inhibition Enzyme-Linked Immunoassay for the Detection of Paralytic Shellfish Toxins. In: E. Graneli, B. Sundstroem, L. Elder and D.M. Anderson (eds). Toxic Marine Phytoplankton, Conference Proceedings - International Conference on Toxic Marine Phytoplankton, Sweden, June 1989, pp. 339-334.
- Chai, T-J., Han, T-J. and Cockey, R. R., 1994. Microbiological quality of shellfish-growing waters in Chesapeake Bay. *Journal of Food Protection*, 57 (3): 229-234.
- Chang, J. C. H., Ossoff, S. F., Lobe, D. C., Dorfman, M. H., Dumais, C. M., Qualls, R. G. and Johnson, J. D., 1985. UV inactivation of pathogenic and indicator microorganisms. *Applied and Environmental Microbiology*, 49 (6): 1361-1365.
- Chang, P. W., Liu, O. C., Miller, L. T. and Li, S. M., 1971. Multiplication of human enteroviruses in northern quahogs. *Proceedings of the Society of Experimental Biology and Medicine*, 136: 1380-1384.
- Chen, C. H. and Chang, T. C., 1995. An enzyme-linked immunosorbent assay for the rapid detection of *Vibrio parahaemolyticus*. *Journal of Food Protection*, 58 (8): 873-878.
- Chung, H., Jaykus, L., and Sobsey, M D., 1996. Detection of human enteric viruses in oysters by in vivo and in vitro amplification of nucleic acids. *Applied Environmental Microbiology*, 62 (10): 3772-3778.
- Clover, D.O., 1994a. Viral foodborne disease agents of concern. *Journal of Food Protection*, 57 (2): 176-178.
- Clover, D.O., 1994b. Epidemiology of viral foodborne disease. *Journal of Food Protection*, 57 (3): 263-266.
- Cole, M. T., Kilgen, M. B., Reily, L. A. and Hackney, C. R., 1986. Detection of enteroviruses and bacterial indicators and pathogens in Louisiana oysters and their overlying waters. *Journal of Food Protection*, 49 (8): 596-601.
- Colt, J.E. and Armstrong, D.A., 1981. Nitrogen toxicity to crustaceans, fish, and molluscs. In: L. J. Allen and E. C. Kinney (eds). *Proceedings of the Bioengineering Symposium for Fish Culture*. Fish Culture Section of the American Fisheries Society (FCS Publication 1), pp. 34-47.
- Cook, D. W., 1994. Effect of time and temperature on multiplication of *Vibrio vulnificus* in postharvest Gulf Coast shellstock oysters. *Applied and Environmental Microbiology*, 60 (9): 3483-3484.
- Cook, D. W. and Ellender, R. D., 1986. Relaying to decrease the concentration of oyster-associated pathogens. *Journal of Food Protection*, 49 (3): 196-202.

- Cook, D. W. and Ruple, A.D., 1992. Cold storage and mild heat treatment as processing aids to reduce the numbers of *Vibrio vulnificus* in raw oysters. *Journal of Food Protection*, 55 (12): 985-989.
- Cooper, R.J. and Langlois, D., 1982. Heavy metals in Tasmanian shellfish. *Journal of Applied Toxicology*, 2 (2): 99-109.
- Cromeans, T. L., Nainan, O. V. and Margolis, H. S., 1997. Detection of hepatitis A virus RNA in oyster meat. *Applied and Environmental Microbiology*, 63 (6): 2460-2463.
- Crowther, J.R., 1995. ELISA Theory and Practice. *Methods in Molecular Biology* vol. 42, Humana Press, New Jersey, USA, 223 pp.
- de Mesquita, M., M., F., Evison, L., M. and West, P. A., 1991. Removal of faecal indicator bacteria and bacteriophages from the common mussel (*Mytilus edulis*) under artificial depuration conditions. *Journal of Applied Bacteriology*, 70: 495-501.
- Deng, M.Y., Day, S.P. and Cliver, D. O., 1994. Detection of hepatitis A virus in environmental samples by antigen-capture PCR. *Applied and Environmental Microbiology*, 60 (6): 1927-1933.
- DePaola, A., Capers, G. M. and Alexander, D., 1994. Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf Coast. *Applied and Environmental Microbiology*, 60 (3): 984-988.
- Desenclos, J.C., 1991. A multistate outbreak of hepatitis A caused by consumption of raw oysters. *American Journal of Public Health*, 81: 1268-1272.
- Desmarchelier, P. M., 1989a. *Vibrio cholerae* and other vibrios. In: K. A. Buckle, J. A. Davey, M. J. Eyles, A. D. Hocking, K. G. Newton and E. J. Stuttard (eds). *Foodborne Microorganisms of Public Health Significance*, 4<sup>th</sup> ed. AIFST (NSW Branch) Food Microbiology Group, Sydney, NSW, Australia, pp. 167-176.
- Desmarchelier, P. M., 1989b. *Vibrio parahaemolyticus*. In: K. A. Buckle, J. A. Davey, M. J. Eyles, A. D. Hocking, K. G. Newton and E. J. Stuttard (eds). *Foodborne Microorganisms of Public Health Significance*, 4<sup>th</sup> ed. AIFST (NSW Branch) Food Microbiology Group, Sydney, NSW, Australia, pp. 151-166.
- Di Girolamo, R., Liston, J. and Matches, J., 1975. Uptake and elimination of poliovirus by West Coast oysters. *Applied Microbiology*, 29 (2): 260-264.
- Di Girolamo, R., Liston, J. and Matches, J., 1977. Ionic bonding, the mechanism of viral uptake by shellfish mucus. *Applied and Environmental Microbiology*, 33 (1): 19-25.
- Dienstag, J. L., Gust, I. D., Lucas, C. R., Wong, D. C. and Purcell, R. H., 1976. Mussel-associated viral hepatitis, type A: serological confirmation. *Lancet*, 1: 561-563.
- Dohra, H., Fujishima, M., Fok, A. K. and Allen, R. D., 1994. Monoclonal antibody to a bacterial endonuclear symbiont *Holospora* cross-reacts with proteins of contractile vacuole radial canals of *Paramecium* spp. *Journal of Eukaryotic Microbiology*, 41 (5): 503-510.
- Doré, W. J. and Lees, D. N., 1995. Behaviour of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. *Applied and Environmental Microbiology*, 61 (8): 2830-2834.
- Dressel, D. M. and Snyder, M. I., 1991. Depuration - the regulatory perspective. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Depuration*, CRC Press, Boca Raton, FL, pp. 19-23.
- Dupont, J., Ménard, C., Hervé, C. and Minier, B., 1994. Analytical procedure for use of conductance measurement to estimate *Escherichia coli* in shellfish. *Journal of Applied Bacteriology*, 77: 296-302.

- European Council Directive 91/494/EC. Food Safety (Live Bivalve Molluscs and Other Shellfish) Regulations 1992.
- Eyles, M. J., 1980. Accumulation and elimination of viruses by oysters. *Food Technology in Australia*, 32 (2): 89-91.
- Eyles, M. J., 1986. Transmission of viral disease by food: an update. *Food Technology in Australia*, 38 (6): 239-250.
- Eyles, M. J. and Davey, G. R., 1984. Microbiology of commercial depuration of the Sydney Rock oyster, *Crassostrea commercialis*. *Journal of Food Protection*, 47 (9): 703-706.
- Eyles, M. J. and Davey, J. A., 1989. Enteric indicator organisms. In: K. A. Buckle, J. A. Davey, M. J. Eyles, A. D. Hocking, K. G. Newton and E. J. Stuttard (eds). *Foodborne Microorganisms of Public Health Significance*, 4<sup>th</sup> ed. AIFST (NSW Branch) Food Microbiology Group, Sydney, NSW, Australia, pp. 25-50.
- Eyles, M. J., Davey, G. R. and Huntley, E. J., 1981. Demonstration of viral contamination of oysters responsible for an outbreak of viral gastroenteritis. *Journal of Food Protection*, 44 (4): 294-296.
- Fayer, R., Farley, C. A., Lewis, E. J., Trout, J. M. and Graczyk, T. K., 1997. Potential role of the Eastern oyster, *Crassostrea virginica*, in the epidemiology of *Cryptosporidium parvum*. *Applied and Environmental Microbiology*, 63 (5): 2086-2088.
- Fleet, G. H., 1978. Oyster depuration - a review. *Food Technology in Australia*, 30: 444-454.
- Fleet, G. and Grohmann, G., 1993. Modern methods in microbiological analysis. In: L. Gilbert, R. Robins-Browne, D. Groot Obbink, M. Eyles, P. Wood and K. Murray (eds). *Recent Advances in Microbiology* vol. 1. National Examinations Board the Australian Society for Microbiology Inc. Melbourne, pp. 259-299.
- Frankish, K., 1989. Oyster hatchery program. In: J.A. Nell (ed.), *Collected Open Day Booklets*, Brackish Water Fish Culture Research Station 1973 to 1988. NSW Agriculture and Fisheries, Salamander Bay, pp. 466-469.
- Fremy, J. M., Ledoux, M., Bilodeau, M., Major, M., Murail, I., and Jamet, J., 1993. Occurrence of paralytic phytotoxins in scallops from Asia. *Sciences des Aliments*, 13: 751-759.
- Gabric, A. J. and Bell, P. R. F., 1993. Review of the effects of non-point nutrient loading on coastal ecosystems. *Australian Journal of Freshwater Research*, 44: 261-283.
- Gill, O.N., Cubitt, W.D., McSwaggan, N.A., Watney, B.M. and Bartlett, C.L.R. 1983. Epidemic of gastroenteritis caused by oysters contaminated with small, round structured viruses. *British Medical Journal*, 287: 1532-1534.
- Gippel, C. J. 1995. Potential of turbidity monitoring for measuring the transport of suspended solids in streams. *Hydrological Processes*, 8: 83-97.
- Gippel, C.J. 1983. The Effect of Water Colour, Particle Size and Particle Composition on Stream Water Turbidity Measurements, Working Paper 1988/3, Department of Geography and Oceanography, University College, Australian Defence Force Academy, Canberra, 38 pp.
- Goyal, S. M., Adams, W. N., O'Malley, M. L. and Lear, D. W., 1984. Human pathogenic viruses at sewage sludge disposal sites in the Middle Atlantic region. *Applied and Environmental Microbiology*, 48 (4): 758-763.
- Goyal, S. M., Gerba, C. P. and Melnick, J. L., 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. *Applied and Environmental Microbiology*, 34 (2): 139-149.

- Goyal, S. M., Gerba, C. P. and Melnick, J. L., 1979. Human enteroviruses in oysters and their overlying waters. *Applied and Environmental Microbiology*, 37 (3): 572-581.
- Grabow, W. O. K., de Villiers, J. C. and Prinsloo, N., 1991. An Assessment of Methods for the Microbiological Analysis of Shellfish. In: W. O. K. Grabow, R. Morris and K. Botzenhart (eds). *Health-Related Water Microbiology. Proceedings of an International Symposium organised by the IAWPRC Specialist Group on Health-Related Water Microbiology - Tübingen, Germany, 1-6 April 1990.* Water Science and Technology, 24: 413-416.
- Grau, F. H., 1989. *Salmonella: Physiology, Pathogenicity and Control.* In: K. A. Buckle, J. A. Davey, M. J. Eyles, A. D. Hocking, K. G. Newton and E. J. Stuttard (eds). *Foodborne Microorganisms of Public Health Significance*, 4<sup>th</sup> ed. AIFST (NSW Branch) Food Microbiology Group, Sydney, NSW, Australia, pp. 83-98.
- Grohmann, G., 1997. Viruses, Food and Environment. In K. A. Buckle, J. A. Davey, M. J. Eyles, A. D. Hocking, K. G. Newton and E. J. Stuttard (eds), *Foodborne Microorganisms of Public Health Significance*. 5<sup>th</sup> ed. AIFST (NSW) Food Microbiology Group, Sydney, NSW Australia pp. 551- 570.
- Grohmann, G. S., Murphy, A. M., Christopher, P. J., Auty, E. and Greenberg, H. B., 1981. Norwalk virus gastroenteritis in volunteers consuming depurated oysters. *Australian Journal of Experimental Biology and Medical Science*, 59: 219-28.
- Groubert, T. N. and Oliver J. D., 1994. Interaction of *Vibrio vulnificus* and the Eastern Oyster, *Crassostrea Virginica*. *Journal of Food Protection*, 57 (3): 224-228.
- Guzewich, J. J. and Morse, D. L., 1986. Sources of shellfish in outbreaks of probable viral gastroenteritis: implications for control. *Journal of Food Protection*, 49 (5): 389-394.
- Hackney, C. R. and Pierson, M. D. (eds), 1994. *Environmental Indicators and Shellfish Safety.* Chapman and Hall, New York, 523 pp.
- Hagen, C. J., Sloan, E. M., Lancette, G. A., Peeler, J. T. and Sofos, J. N., 1994. Enumeration of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various seafoods with two enrichment broths. *Journal of Food Protection*, 57 (5): 403-409.
- Hall, S., 1991. Natural Toxins. In: D. R. Ward and C. R. Hackney (eds). *Microbiology of Marine Food Products*, Van Nostrand Reinhold, New York, pp. 301-330.
- Hallegraeff, G. M., Stanley, S. O., Bolch, C. J. and Blackburn, S. I., 1989. *Gymnodinium catenatum* Blooms and Shellfish Toxicity in Southern Tasmania. In: T. Okaichi, D. M. Anderson and T. Nemoto (eds). *Red Tides: Biology, Environmental Science and Toxicology*, Elsevier, North Holland, pp. 77-80.
- Hamblet, F. E., Hill, W. F., Akin, E. W. and Benton, W. H., 1969. Oysters and human viruses: effect of seawater turbidity on Poliovirus uptake and elimination. *American Journal of Epidemiology*, 89 (5): 562-571.
- Hansen, W., Freney, J., Benyagoub, H., Letouzey, M. N., Gigi, J., and Wauters, G. 1993. Severe human infections caused by *Vibrio metschnikovii*. *Journal of Clinical Microbiology*, 31 (9): 2529-2530.
- Hardiman, S. and Pearson, B., 1995. Heavy metals, TBT and DDT in the Sydney rock oyster (*Saccostrea commercialis*) sampled from the Hawkesbury River estuary, NSW, Australia. *Marine Pollution Bulletin*, 30 (8): 563-567.
- Harris-Young, L., Tamplin, M. L., Fisher, W. S. and Mason, J. W., 1993. Effects of physiochemical factors and bacterial colony morphotype on association of *Vibrio vulnificus* with haemocytes of *Crassostrea virginica*. *Applied and Environmental Microbiology*, 59 (4): 1012-1017.

- Harris-Young, L., Tamplin, M. L., Mason, J. W., Aldrich, H. C. and Jackson, J. K., 1995. Viability of *Vibrio vulnificus* in association with haemocytes of the American oyster (*Crassostrea virginica*). *Applied and Environmental Microbiology*, 61 (1): 52-57.
- Havelaar, A., Furuse, K. and Hogeboom, W. M., 1986. Bacteriophages and indicator bacteria in human and animal faeces. *Journal of Applied Bacteriology*, 60 :255-262.
- Havelaar, A.H., Nieuwstad, Th. J., Meulemans, C.C.E., and van Olphen, M., 1991. F-specific RNA bacteriophages as model viruses in UV disinfection of wastewater. *Water Science Technology*, 24 (2): 347-352.
- Hay, B. and Scotti, P., 1986. Evidence for intracellular absorption of virus by the Pacific oyster, *Crassostrea gigas*. *New Zealand Journal of Marine and Freshwater Research*, 20: 655-659.
- Herrington, T., 1991. Use of ultraviolet light in depuration In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Depuration* CRC Press, Boca Raton, FL, pp. 137-144.
- Hill, W. F., Hamblett, F. E. and Akin, E. W., 1967. Survival of Poliovirus in flowing turbid sea water treated with ultraviolet light. *Applied Microbiology*, 15 (3): 533-536.
- Hill, W. F., Hamblett, F. E. and Benton, W. H., 1969. Inactivation of Poliovirus type 1 by the Kelly-Purdy ultraviolet sea water treatment unit. *Applied Microbiology*, 17: 1-6.
- Hill, W. F., Hamblett, F. E., Benton, W. H. and Akin, E. W., 1970. Ultraviolet devitalisation of eight selected enteric viruses in estuarine water. *Applied Microbiology*, 19 (5): 805-812.
- Hoff, J. C. and Becker, R. C., 1969. The accumulation and elimination of crude and clarified Poliovirus suspensions by shellfish. *American Journal of Epidemiology*, 90: 53-61.
- Holliday, J. E., 1995. Nursery culture of Sydney rock oysters, *Saccostrea commercialis* (Iredale & Roughley, 1933) and Pacific oysters, *Crassostrea gigas* (Thunberg, 1793). Unpublished PhD thesis, University of Tasmania, Australia, 288 pp.
- Holliday, J. E. and Goard, L. J., 1986. Survey of Sydney rock oyster larvae settlement at Port Stephens, NSW. *Australian Fisheries*, 45 (3): 27-28.
- Honkanen, R. E., Mowdy, D.E. and Dickey, R. W., 1996. Detection of DSP-toxins, okadaic acid and dinophysis toxin-1 in shellfish by serine/threonine protein phosphatase assay. *Journal of AOAC International*, 79 (6): 1336-1343.
- Huguenin, J. E. and Colt, J., 1989. Design and Operating guide for Aquaculture Seawater Systems. *Developments in Aquaculture and Fisheries Science*, 20: 170-171.
- Hughes-Games, W. L., 1977. Growing the Japanese oyster, *Crassostrea gigas*, in subtropical seawater ponds. 1. Growth rate, survival and quality index. *Aquaculture*, 11: 217-229.
- Hungerford, J. M., and Wekell, M. M., 1992 . Analytical Methods for Marine Toxins. In: J.M. Hungerford, M.M. Wekell and A.T.Tu (eds). *Food-poisoning, Handbook of Natural Toxins Volume 7*. Marcel Dekker, New York, USA, pp. 416-473.
- Huq, A., Colwell, R. R., Rahman, R., Ali, A., Chowdhury, M. A. R., Parveen, S., Sack, D. A. and Russek-Cohen, E., 1990. Detection of *V. cholerae* O1 in the aquatic environment by fluorescent monoclonal antibody and culture method. *Applied and Environmental Microbiology*, 56 (8): 2370-2373.
- Idema, G. K., Bateman, B. W., Kfir, R. and Grabow, W. O. K., 1991. A Comparison of Methods for the Isolation of a Wide Range of Viruses from Shellfish. In: W. O. K.

- Grabow, R. Morris and K. Botzenhart (eds). Health-Related Water Microbiology. Proceedings of an International Symposium organised by the IAWPRC Specialist Group on Health-Related Water Microbiology - Tubingen, Germany, 1-6 April, 1990. Water Science and Technology, 24 (2): 427-430.
- Jaykus, L. A., Hemmard, M. T. and Sobsey, M. D., 1994. Human Enteric Pathogenic Viruses. In: C. R. Hackney and M. D. Pierson (eds). Environmental Indicators and Shellfish Safety. Chapman and Hall, New York, pp. 92-153.
- Jinneman, K. C., Trost, P. A., Hill, W. E., Weagant, S. D., Bryant, J. D., Kaysner, C. A. and Wekell, M. M., 1995. Comparison of template preparation methods from foods for amplification of *Escherichia coli* O157 Shiga-like toxins type I and II DNA by multiplex polymerase chain reaction. Journal of Food Protection, 58 (7): 722-726.
- Joanny, M., Belin, C., Claisse, D., Miossec, L., Berthome, J-P., Grouhel, A. and Raffin, B., 1993. Quality of the Marine Coastal Environment. Les Editions de l'IFREMER, Brest, 240 pp.
- Jones, D. D., Law, R. and Bej, A. K., 1993. Detection of *Salmonella* spp in oysters using polymerase chain reactions (PCR) and gene probes. Journal of Food Science, 58 (6): 1191-1197.
- Jones, S. H., Howell, T. L. and O' Neill, K. R., 1991. Differential elimination of indicator bacteria and pathogenic *Vibrio* sp. from eastern oysters (*Crassostrea virginica* Gmelin, 1791) in a commercial controlled purification facility in Maine. Journal of Shellfish Research, 10 (1): 105-111.
- Kator, H. and Rhodes, M. W., 1991. Indicators and alternate indicators of growing water quality. In: D. R. Ward and C. R. Hackney (eds). Microbiology of Marine Food Products, Van Nostrand Reinhold, New York, pp. 135-196.
- Kator, H. and Rhodes, M., 1994. Microbial and chemical indicators. In: C. R. Hackney and M. D. Pierson (eds). Environmental Indicators and Shellfish Safety. Chapman shellfish and Hall, New York, pp. 30-91.
- Kaysner, C. A., Abeyta, C., Jr., Jinneman, K. C. and Hill, W. E., 1994a. Enumeration and differentiation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* by DNA-DNA colony hybridization using the hydrophobic grid membrane filtration technique for isolation. Journal of Food Protection, 57 (2): 163-165.
- Kaysner, C. A., Abeyta, C., Jr., Trost, P. A., Wetherington, J. H., Jinneman, K. C., Hill, W. E. and Wekell, M. M., 1994b. Urea hydrolysis can predict the potential pathogenicity of *Vibrio parahaemolyticus* strains isolated in the Pacific Northwest. Applied and Environmental Microbiology, 60 (8): 3020-3022.

- Kaysner, C. A., Tamplin, M. L., Wekell, M. M., Stott, R. F. and Colburn, K. G., 1989. Survival of *Vibrio vulnificus* in shellstock and shucked oysters (*Crassostrea gigas* and *Crassostrea virginica*) and effect of isolation medium on recovery. *Applied and Environmental Microbiology*, 55 (12): 3072-3079.
- Kelly, G. J. and Hallegraef, G. M., 1992. Dinoflagellate Toxins in Australian Shellfish. In: D. Watters, M. Lavin, D. Maguire and J. Pearn (eds). *Toxins and Targets: Effects of Natural and Synthetic Poisons on Living Cells and Fragile Ecosystems*, Harwood Academic Publishers, Chur, Switzerland, pp. 1-9.
- Kelly, M. T., Hickman-Brenner, F. W. and Farmer, J. J., 1991. *Vibrio*. In: A. Balows, W. J. Hausler, K. L. Herrmann, H. D. Isenberg and H. J. Shadomy (eds). *Manual of Clinical Microbiology*, 5<sup>th</sup> ed. American Society for Microbiology, Washington D.C. USA, pp 384-395.
- Kfir, R., Burger, J. S. and Idema, G. K., 1993. Detection of *Salmonella* in shellfish grown in polluted sea water. *Water Science and Technology*, 27 (3-4): 41-44.
- Kirk, J. T. O., 1986. Optical Limnology – a Manifesto. In: W. D. Williams and P. De Deckker (eds). *Limnology in Australia*, CSIRO Melbourne, pp 33-62.
- Klontz, K. C. and Rippey, S., 1991. Epidemiology of Molluscan-Borne Illnesses. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Depuration*, CRC Press, Boca Raton, FL, pp. 47-58.
- Klontz, K. C., Williams, L., Baldy, L. M. and Campos, M., 1993. Raw oyster-associated *Vibrio* infections: Linking epidemiologic data with laboratory testing of oysters obtained from a retail outlet. *Journal of Food Protection*, 56 (11): 977-979.
- Koch, W. H., Payne, W. L., Wentz, B. A. and Cebula, T. A., 1993. Rapid polymerase chain reaction method for detection of *Vibrio cholerae* in foods. *Applied and Environmental Microbiology*, 59 (2): 556-560.
- Koff, R. S., Grady, G. F., Chalmers, T. C., Mosley, J. W. and Swartz, B. L., 1967. Viral hepatitis in a group of Boston hospitals. 3. Importance of exposure to shellfish in a nonepidemic period. *New England Journal of Medicine*, 276 (13): 703-710.
- Kohn, M. A., Farley, T. A., Ando, T., Curtis, M., Wilson, S. A., Jin, Q., Monroe, S. S., Baron, R. C., McFarland, L. M. and Glass, R. I., 1995. An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters. Implications for maintaining safe oyster beds. *Journal of the American Medical Association*, 273 (6): 466-471. [Published erratum appears in *JAMA* 1995 May 17; 273 (19): 1492].
- Kreger, A., DeChatelet, L. and Shirley, P., 1981. Interaction of *Vibrio vulnificus* with human polymorphonuclear leucocytes: association of virulence with resistance to phagocytosis. *The Journal of Infectious Diseases*, 144 (3): 244-248.
- Kueh, S.W., and Chan, K., 1985. Bacteria in bivalve shellfish with special reference to the oyster. *Journal of Applied Bacteriology*, 59: 41-47.
- Kvenberg J. E., 1991. Nonindigenous bacterial pathogens. In: D. R. Ward and C. Hackney (Eds). *Microbiology of Marine Food Products*, Van Nostrand Reinhold, New York, pp. 267-284.
- LaBelle, R. L. and Gerba, C. P., 1980. Influence of estuarine sediment on virus survival under field conditions. *Applied and Environmental Microbiology*, 39 (4): 749-755.
- Landry, E. F., Vaughn, J. M., Vicale, T. J. and Mann, R., 1982. Inefficient accumulation of low levels of monodispersed and faeces-associated Poliovirus in oysters. *Applied and Environmental Microbiology*, 44 (6): 1362-1369.

- Landry, E. F., Vaughn, J. M., Vicale, T. J. and Mann, R., 1983. Accumulation of sediment-associated viruses in shellfish. *Applied and Environmental Microbiology*, 45 (1): 238-247.
- Langdon, C. J. and Newell, R. I. E., 1996. Digestion and nutrition in larvae and adults. In: V. S. Kennedy, R. I. E. Newell and A. F. Elbe (eds). *The Eastern Oyster Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland, pp. 231-269.
- Laycock, M.V, Kralovec J., and Richards, R., 1997. A simple procedure for sulfation and <sup>35</sup>S radiolabelling of paralytic shellfish poisoning (PSP) gonyautoxins. *Natural Toxins*, 5: 36-42.
- Legan, R.W., 1982. Ultraviolet light takes on a CPI role. *Chemical Engineering*, 89 (2): 95-100.
- Le Guyader, F., Apaire-Marchais, V., Brillet, J. and Billaudel, S., 1993. Use of genomic probes to detect hepatitis A virus and enterovirus RNAs in wild shellfish and relationship of viral contamination to bacterial contamination. *Applied and Environmental Microbiology*, 59 (11): 3963-3968.
- Le Paulouë, P., Langlais, B., Poggi, R. and Perrot, Y., 1991. French Shellfish Industry Regulatory Status and Depuration Techniques. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds) *Molluscan Shellfish Depuration*, CRC Press, Boca Raton, FL, pp. 341-360.
- LeChevalier, M. W., Evans, T. M. and Seidler, R. J., 1981. Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. *Applied and Environmental Microbiology*, 42 (1): 159-167.
- Lee, C. Y., Pan, S. F. and Chen, C. H., 1995. Sequence of a cloned pR72H fragment and its use for detection of *Vibrio parahaemolyticus* in shellfish with the PCR. *Applied and Environmental Microbiology*, 61 (4): 1311-1317.
- Lester, R. J. G., 1989. Diseases of molluscs. In: *Invertebrates in Aquaculture*. D. I. Brydon (ed). Post Graduate Committee in Veterinary Science, University of Sydney, pp. 129-142.
- Lewis, G., Loutit, M. W. and Austin, F. J., 1986. Enteroviruses in mussels and marine sediments and depuration of naturally accumulated viruses by green lipped mussels (*Perna canaliculus*). *New Zealand Journal of Marine and Freshwater Research*, 20: 431-437.
- Lightfoot, N. F., Tillett, H. E., Boyd, P. and Eaton, S., 1994. Duplicate split samples for internal quality control in routine water microbiology. *Letters in Applied Microbiology*, 19: 321-324.
- Linco, S. J. and Grohmann, G. S., 1980. The Darwin outbreak of oyster-associated viral gastroenteritis. *Medical Journal of Australia*, 1: 211-213.
- Lindenauer, K.G. and Darby, J.L., 1994. Ultraviolet disinfection of wastewater: effect of dose on subsequent photoreactivation. *Water Research Oxford*, 28 (4): 805-817.
- Liu, O. C., Seraichekas, H. R. and Murphy, B. L., 1966a. Fate of Poliovirus in Northern Quahogs. *Proceedings of the Society for Experimental Biology and Medicine*, 121: 601-607.
- Liu, O. C., Seraichekas, H. R. and Murphy, B. L., 1966b. Viral pollution of shellfish. 1. Some basic facts of uptake. *Proceedings of the Society for Experimental Biology and Medicine*, 123: 481-487.

- Locke, S.J. and Thibault, P., 1994. Improvement in detection limits for the determination of paralytic shellfish poisoning toxins in shellfish tissues using capillary electrophoresis electrospray mass spectrometry and discontinuous buffer systems. *Analytical Chemist*, 66: 3436-3446.
- Maidment, D. R., 1996. To Investigate the Developments and latest Trends in Commercial Shellfish Quality Assurance Programs in Ireland, United Kingdom, France, Netherlands, Canada and North East USA. The Winston Churchill Memorial Trust of Australia, 29 pp.
- Matté, G. R., Matté, M. H., Sato, M. I. Z., Sanchez, P. S., Rivera, I. G. and Martins, M. T., 1994. Potentially pathogenic vibrios associated with mussels from a tropical region on the Atlantic coast of Brazil. *Journal of Applied Bacteriology*, 57: 281-287.
- Mechsner, K. I., Fleischmann, T., Mason, C. A. and Hamer, G., 1991. UV disinfection: Short term inactivation and revival. *Health Related Microbiology*, 24 (2): 339-342.
- Metcalf, T. G. and Stiles, W. C., 1965. The accumulation of enteric viruses by the oyster, *Crassostrea virginica*. *Journal of Infectious Diseases*, 115: 68-76.
- Metcalf, T. G., Melnick, J. L. and Estes, M. K., 1995. Environmental virology. *Annual Review of Microbiology*, 49: 461-487.
- Metcalf, T. G., Mullin, B., Eckerson, D., Moulton, E. and Larkin, E. P., 1979. Bioaccumulation and depuration of enteroviruses by the soft-shelled clam, *Mya arenaria*. *Applied and Environmental Microbiology*, 38 (2): 275-282.
- Miceli, G. A., Watkins, W. D. and Rippey, S. R., 1993. Direct plating procedure for enumerating *Vibrio vulnificus* in oysters (*Crassostrea virginica*). *Applied and Environmental Microbiology*, 59 (11): 3519-3524.
- Miocevic, I., Smith, J., Owens, L. and Speare, R., 1993. Ultraviolet sterilisation of model viruses important to finfish aquaculture in Australia. *Australian Veterinary Journal*, 70: 25-27.
- Millard, J., Appleton, H. and Parry, J. V., 1987. Studies on heat inactivation of hepatitis A virus with special reference to shellfish. Part 1. Procedures for infection and recovery of virus from laboratory-maintained cockles. *Epidemiology and Infection*, 98: 397-414.
- Morse, D. L., Guzewich, J. J., Hanrahan, J. P., Stricof, R., Shayegani, M., Deibel, R., Grabau, J. C., Nowak, N. A., Herrmann, J. E., Cukor, G. and Blacklow, N. R., 1986. Widespread outbreaks of clam- and oyster-associated gastroenteritis. Role of Norwalk virus. *New England Journal of Medicine*, 314 (11): 678-681.
- Morton, S.L., and Tindall, D. R., 1996. Determination of okadaic acid content of dinoflagellate cells: a comparison of the HPLC-fluorescent method and two monoclonal antibody ELISA test kits. *Toxicon*, 34 (8): 947-954.
- Motes, M. L. and Peeler, J. T., 1991. Field evaluation of the MUG assay for enumerating *Escherichia coli* in sea water and oysters from southeastern United States. *Journal of Food Protection*, 54 (4): 246-248.
- Motes, M., DePaola, A., Zywno-Van Ginkel, S. and McPhearson, M., 1994. Occurrence of toxigenic *Vibrio cholerae* O1 in oysters in Mobile Bay, Alabama: An ecological investigation. *Journal of Food Protection*, 57 (11): 975-980.

- Muntada-Garriga, J. M., Rodriguez-Jerez, J. J., Lopez-Sabater, E. I. and Mora-Ventura, M. T., 1995. Effect of chill and freezing temperatures on survival of *Vibrio parahaemolyticus* inoculated in homogenates of oyster meat. *Letters in Applied Microbiology*, 20: 225-227.
- Murphree, R. L. and Tamplin, M. L., 1995. Uptake and retention of *Vibrio cholerae* O1 in the eastern oyster, *Crassostrea virginica*. *Applied and Environmental Microbiology*, 61 (10): 3656-3660.
- Murphy, A. M., Grohmann, G. S., Christopher, P. J., Lopez, W. A., Davey, G. R. and Millsom, R. H., 1979. An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. *Medical Journal of Australia*, 2: 329-333.
- Nell, J. A., 1985. Effects of temperature, salinity and fluorine on oysters. In: *Collected Open Day Booklets of the Brackish Water Fish Culture Research Station 1973–1988*, NSW Agriculture and Fisheries, pp. 342-344.
- Nell, J. A., 1992. Oyster nutrition. In: Allan G. L. and Dall W. (eds). *Proceedings Aquaculture Nutrition Workshop, Salamander Bay, 15-17 April 1991*. Brackish Water Fish Culture Research Station, Salamander Bay, Australia, pp. 147-155.
- Nell, J. A. and Chvojka, R., 1992. The effect of bis-tributyltin oxide (TBTO) and copper on the growth of juvenile Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) and Pacific oysters *Crassostrea gigas* Thunberg. *The Science of the Total Environment*, 125: 193-201.
- Nell, J. A. and Holliday, J. E., 1988. Effect of salinity on the growth and survival of Sydney rock oyster (*Saccostrea commercialis*) and Pacific oyster (*Crassostrea gigas*) larvae and spat. *Aquaculture*, 68: 39-44.
- Nell, J. A. and Livanos, G., 1988. Effects of fluoride concentration in seawater on growth and fluoride accumulation by Sydney rock oyster (*Saccostrea commercialis*) and flat oyster (*Ostrea angasi*) spat. *Water Research*: 22 (6): 749-753.
- Newell, R. I. E. and Langdon, C. J., 1996. Mechanisms and physiology of larval and adult feeding. In: V. S. Kennedy, R. I. E. Newell and A. F. Elbe (eds). *The Eastern Oyster Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland, pp. 185-229.
- New South Wales EPA (Environmental Protection Agency), 1996. Organochloride and trace metal contamination in NSW wild oysters. Draft paper.
- Nishio, T., Nakamori, J. and Miyazaki, K., 1981. Survival of *Salmonella typhi* in oysters. *Zentralblatt für Bakteriologie und Hygiene*, 172: 415-426.
- National Shellfish Sanitation Program (NSSP), 1990a. *Manual of Operations, Part 1: Sanitation of Shellfish-Growing Areas*. Public Health Service, U. S. Food and Drug Administration, Washington D.C.
- National Shellfish Sanitation Program (NSSP), 1990b. *Manual of Operations, Part 2: Sanitation of Harvesting, Processing and Distribution of Shellfish*. Public Health Service, U. S. Food and Drug Administration, Washington D.C.
- National Shellfish Sanitation Program (NSSP), 1995a. *Manual of Operations, Part 1: Sanitation of Shellfish-Growing Areas*. US Department of Health and Human Services Public Health Service, U. S. Food and Drug Administration, Washington D.C.
- National Shellfish Sanitation Program (NSSP), 1995b. *Manual of Operations, Part 2: Sanitation of Harvesting, Processing and Distribution of Shellfish*. US Department of Health and Human Services Public Health Service, U. S. Food and Drug Administration, Washington D.C.
- NSW Food Act, No. 231, 1989. NSW Government, Sydney, 63 pp.

- O'Hara, P.J., 1993. Overview of the marine biotoxin crisis in 1993. In: J. A. Jasperse (ed.). *Marine Toxins and New Zealand Shellfish. Proceedings of a Workshop on Research Issues*, 10-11 June 1993. The Royal Society of New Zealand, Miscellaneous Series, 24, pp. 3-6.
- Oliver, J. D. and Bockian, R., 1995. In vivo resuscitation, and virulence towards mice, of viable but nonculturable cells of *Vibrio vulnificus*. *Applied and Environmental Microbiology*, 61 (7): 2620-2623.
- Oliver, J. D., Hite, F., McDougal, D., Andon, N. and Simpson, L. M., 1995. Entry into, and resuscitation from, the viable but nonculturable state by *Vibrio vulnificus* in an estuarine environment. *Applied and Environmental Microbiology*, 61 (7): 2624-2630.
- Oliver, J. D., Nilsson, L. and Kjelleberg, S., 1991. Formation of nonculturable *Vibrio vulnificus* cells and its relationship to the starvation state. *Applied and Environmental Microbiology*, 57 (9): 2640-2644.
- O'Neill, K. R., Jones, S. H. and Grimes, D. J., 1992. Seasonal incidence of *Vibrio vulnificus* in the Great Bay estuary of New Hampshire and Maine. *Applied and Environmental Microbiology*, 58 (10): 3257-3262.
- O'Sullivan, D. and Kiley, T., 1996. Status of Australian aquaculture 1994/1995. *Austasia Aquaculture Trade Directory*, 1996: 2-12.
- Otwell, W. S., Rodrick, G. E. and Martin, R. E. (eds), 1991. *Molluscan Shellfish Depuration*. CRC Press, Boca Raton, FL, 384 pp.
- Park, D.L., 1995. Prediction of aquatic biotoxin potential in fish and shellfish harvesting areas: ciguatera and diarrhoeic shellfish poisoning. In: N. Della-Croce, S. Connell, R. Abels (eds). *Coastal Ocean Space Utilisation. 3 International Symposium on Coastal Ocean Space Utilisation. Marginal Seas: Problems and Opportunities*. Genova (Italy), April 1993. pp. 271-282.
- Parry, G. D., Langdon, J. S. and Huisman, J. M., 1989. Toxic effects of a bloom of a diatom *Rhizosolenia chunii* on shellfish in Port Phillip Bay, south-eastern Australia. *Marine Biology*, 102: 25-41.
- Parker, R. S. and Lewis, D. H., 1995. Sandwich enzyme-linked immunosorbent assay for *Vibrio vulnificus* haemolysin to detect *V. vulnificus* in environmental specimens. *Applied and Environmental Microbiology*, 61 (2): 476-480.
- Peeters, J. E., Mazás, E. A., Masschelein, W. J., De Maturana, I. V. M. and Debacker, E., 1989. Effect of disinfection of drinking water with ozone or chlorine dioxide on survival of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, 55 (6): 1519-1522.
- Pilkington, N. H., 1995a. Disinfection of Water and Waste Water by Ultraviolet Radiation. In: L. O. Kolarik and A. J. Priestley (eds). *Modern Techniques in Water and Wastewater Treatment*, CSIRO Publishing, East Melbourne, pp. 81-87.
- Pilkington, N. H., 1995b. Disinfection: an Overview. In: L. O. Kolarik and A. J. Priestley (eds). *Modern Techniques in Water and Wastewater Treatment*, CSIRO Publishing, East Melbourne, pp. 75-79.
- Plusquellec, A., Beucher, M., Le Lay, C., Gueguen, D. and Le Gal, Y., 1994. Uptake and retention of *Salmonella* by bivalve shellfish. *Journal of Shellfish Research*, 13 (1): 221-227.
- Portnoy, B. L., Mackowiak, P. A., Caraway, C. T., Walker, J. A., McKinley, T. W. and Klein, C. A., 1975. Oyster-associated Hepatitis. *Journal of the American Medical Association*, 233: 1065-1068.

- Power, U. F. and Collins, J. K., 1989. Differential depuration of poliovirus, *Escherichia coli*, and a coliphage by the common mussel, *Mytilus edulis*. *Applied and Environmental Microbiology*, 55 (6): 1386-1390.
- Power, U. F. and Collins, J. K., 1990. Tissue distribution of a coliphage and *Escherichia coli* in mussels after contamination and depuration. *Applied and Environmental Microbiology*, 56 (3): 803-807.
- Quadri, R. B., Buckle, K. A. and Edwards, R. A., 1975. Sewage pollution in oysters grown in the Georges River-botany Bay area. *Food Technology in Australia*, 27: 236-237, 239-242.
- Rao, V. C., Seidel, K. M., Goyal, S. M., Metcalf, T. G. and Melnick, J., 1984. Isolation of Enteroviruses from water, suspended solids, and sediments from Galveston Bay: survival of Poliovirus and Rotavirus adsorbed to sediments. *Applied and Environmental Microbiology*, 48 (2): 404-409.
- Richards, G. P., 1985. Outbreaks of shellfish-associated enteric virus illness in the United States: Requisite for development of viral guidelines. *Journal of Food Protection*, 48 (9): 815-823.
- Richards, G. P., 1988. Microbial purification of shellfish: a review of depuration and relaying. *Journal of Food Protection*, 51 (3): 218-251.
- Rippey, S.R., 1989. Shellfish borne disease outbreaks. Northeast Technical Services Units, Food and Drug Administration, Daisville, R.I.
- Rodrick, G. E. and Schneider, K. R., 1991. Depuration and Relaying of Molluscan Shellfish. In: M. D. Pierson and C. R. Hackney (eds). *Comprehensive Literature Review of Indicators in Shellfish and Their Growing Waters*, Louisiana Universities Consortium, USA, pp 17-1 to 17-47.
- Roesijadi, G., 1996. Environmental factors: Response to metals. In: V. S. Kennedy, R. I. E. Newell and A. F. Elbe (eds), *The Eastern Oyster *Crassostrea virginica**. Maryland Sea Grant College, College Park, Maryland, pp. 515-537.
- Romalde, J. L., Estes, M. K., Szücs, G., Atmar, R. L., Woodley, C. M. and Metcalf, T. G., 1994. In situ detection of hepatitis A virus in cell cultures and shellfish tissues. *Applied and Environmental Microbiology*, 60 (6): 1921-1926.
- Rowse, A. J. and Fleet, G. H., 1982. Viability and release of *Salmonella charity* and *Escherichia coli* from oyster faeces. *Applied and Environmental Microbiology*, 44 (3): 544-548.
- Rowse, A. J. and Fleet, G. H., 1984. Effects of water temperature and salinity on elimination of *Salmonella charity* and *Escherichia coli* from Sydney rock oysters (*Crassostrea commercialis*). *Applied and Environmental Microbiology*, 48 (5): 1061-1063.
- Roy, D., Wong, P. K. Y., Engelbrecht, R. S. and Chian, E. S. K., 1981. Mechanism of enteroviral inactivation by ozone. *Applied and Environmental Microbiology*, 41 (3): 718-723.
- Scotti, P. D., Fletcher, G. C., Buisson, D. H. and Fredericksen, S., 1983. Virus depuration of the Pacific oyster (*Crassostrea gigas*) in New Zealand. *New Zealand Journal of Science* 26: 9-13.
- Seraichekas, H. R., Brashear, D. A., Barnick, J. A., Carey, P. F. and Liu, O. C., 1968. Viral depuration by assaying individual shellfish. *Applied Microbiology* 16 (12): 1865-1871.
- Shangkuan, Y. H., Show, Y. S. and Wang, T. M., 1995. Multiplex polymerase chain reaction to detect toxigenic *Vibrio cholerae* and to biotype *Vibrio cholerae* O1. *Journal of Applied Bacteriology*, 79: 264-273.

- Sharp, D. G., Young, D. C., Floyd, R. and Johnson, J. D., 1980. Effect of ionic environment on the inactivation of poliovirus in water by chlorine. *Applied and Environmental Microbiology*, 39 (3): 530-534.
- Shumway, S. E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. *Journal of the World Aquaculture Society*, 21 (2): 65-104.
- Shumway, S. E., 1996. Natural Environmental Factors. In: V. S. Kennedy, R. I. E. Newell and A. F. Elbe (eds). *The Eastern Oyster *Crassostrea virginica**. Maryland Sea Grant College, College Park, Maryland, pp. 467-513.
- Shumway, S.E. and Cembella, A.D., 1994. Toxic algal blooms: potential hazards to scallop culture and fisheries. *Memoirs of the Queensland Museum*, 36 (2): 361-372.
- Simpson, L. M., White, V. K., Zane, S. F. and Oliver, J. D., 1987. Correlation between virulence and colony morphology in *Vibrio vulnificus*. *Infection and Immunity*, 55 (1): 269-272.
- Sloan, E. M., Hagen, C. J., Lancette, G. A., Peeler, J. T. and Sofos, J. N., 1992. Comparison of five selective enrichment broths and two selective agars for recovery of *Vibrio vulnificus* from oysters. *Journal of Food Protection*, 55 (5): 356-359.
- Smith, E. M., Gerba, C. P. and Melnick, J. L., 1978. Role of sediment in the persistence of Enteroviruses in the estuarine environment. *Applied and Environmental Microbiology*, 35 (4): 685-689.
- Smith, I. R. and Nell. J. A., (year unknown, ≥1991a). The rate of removal of algae by Sydney rock oysters (*Saccostrea commercialis*) at three temperatures. Unpublished NSW Fisheries Report, Salamander Bay, NSW, 2 pp.
- Smith, I. R. and Nell. J. A., (year unknown, ≥1991b). The effect of culling on the algal clearance rate of Sydney rock oysters (*Saccostrea commercialis*). Unpublished NSW Fisheries Report, Salamander Bay, NSW, 4 pp.
- Sobsey, M.D., Davis, A.L. and Rullman, V.A., 1987. Persistence of hepatitis A virus and other viruses in deperated eastern oysters. *Proceedings Oceans 87*, Halifax, Nova Scotia. 5: 1740 -1745.
- Sobsey, M. D. and Jaykus, L-A., 1991. Human enteric viruses and deperation of bivalve molluscs. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Deperation*, CRC Press, Boca Raton, FL, pp. 71-114.
- Sobsey, M. D., Oldham, C. E. and McCall, d. E., 1991. Comparative Inactivation of Hepatitis A Virus and Other Enteroviruses in Water by Iodine. In: W. O. K. Grabow, R. Morris and K. Botzenhart (eds). *Health-related Water Microbiology*. *Proceedings of an International Symposium organised by the IAWPRC Specialist Group on Health-Related Water Microbiology - Tubingen, Germany*, 1-6 April 1990. *Water Science and Technology*, 24 (2): 331-338.
- Son, N. T. and Fleet, G. H., 1980a. Behaviour of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during deperation, re-laying, and storage. *Applied and Environmental Microbiology*, 40 (6): 994-1002.
- Souness, R. A. and Fleet, G. H., 1979. Deperation of the Sydney Rock oyster, *Crassostrea commercialis*. *Food Technology in Australia*, 31: 397-398, 400-404.
- Souness, R. A. and Fleet, G. H., 1991. Bacterial Agents in Shellfish Deperation. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Deperation*, CRC Press, Boca Raton, FL, pp. 59-70.

- Souness, R. A., Bowrey, R. G. and Fleet, G. H., 1979. Commercial depuration of the Sydney rock oyster, *Crassostrea commercialis*. Food Technology in Australia, 31: 531-537.
- Speirs, J. I., Pontefract, R. D. and Harwig, J., 1987. Methods for recovering Poliovirus and Rotavirus from oysters. Applied and Environmental Microbiology, 53 (11): 2666-2670.
- Spotte, 1979. Seawater Aquariums: the Captive Environment. John Wiley, New York, pp. 209-217.
- Stanley, S. and Brown, R., 1988. The Analysis of PSP Toxins in Shellfish. In: L.H. Evans and D. O'Sullivan (eds). Proceedings First Australian Shellfish Aquaculture Conference, October 1988. Curtin University of Technology, Perth, pp. 285-293.
- Stelma, G. N. and McCabe, L. J., 1992. Nonpoint pollution from animal sources and shellfish sanitation. Journal of Food Protection, 55 (8): 649-656.
- Sullivan, J.J., 1988. Methods of analysis for DSP and PSP toxins in shellfish: a review. Journal of Shellfish Research, 7 (4): 587-595.
- Sun, Y. and Oliver, J. D., 1995. Value of cellobiose-polymyxin B-colistin agar for isolation of *Vibrio vulnificus* from oysters. Journal of Food Protection, 58 (4): 439-440.
- Tacket, C. O., Barrett, T. J., Mann, J. M., Roberts, M. A. and Blake, P. A., 1984. Wound infections caused by *Vibrio vulnificus*, a marine vibrio, in inland areas of the United States. Journal of Clinical Microbiology, 19 (2): 197-199.
- Tamplin, M. L. and Capers, G. M., 1992. Persistence of *Vibrio vulnificus* in tissues of Gulf coast oysters, *Crassostrea virginica*, exposed to seawater disinfected with UV light. Applied and Environmental Microbiology, 58 (5): 1506-1510.
- Tamplin, M. L., Martin, A. L., Ruple, A. D., Cook, D. W. and Kaspar, C. W., 1991. Enzyme immunoassay for identification of *Vibrio vulnificus* in seawater, sediment, and oysters. Applied and Environmental Microbiology, 57 (4): 1235-1240.
- Tauxe, R.V., 1997. Emerging foodborne diseases: An evolving public health challenge. Emerging Infectious Diseases, 3 (4): 425-434.
- Tierney, J. T., Sullivan, R., Peeler, J. T. and Larkin, E. P., 1982. Persistence of Polioviruses in shellstock and shucked oysters stored at refrigeration temperature. Journal of Food Protection, 45 (12): 1135-1137.
- Troyan, J. J. and Hansen, S. P., 1989. Treatment of Microbial Contaminants in potable Water Supplies: Technologies and Costs. Pollution Technology Review No. 171, Noyes Data Corporation, New Jersey, USA, 335 pp.
- United States Food and Drug Administration. 1983. England shellfish program review - 1983. Food and Drug Administration, North Kingstown, R.I. pp 68.
- Van Poucke, S. O. and Nelis, H. J., 1997. Limitations of highly sensitive enzymatic presence-absence test for detection of waterborne coliforms and *Escherichia coli*. Applied and Environmental Microbiology, 63 (2): 771-774.
- Vasconcelos, G. J. and Lee, J. S., 1972. Microbial flora of Pacific oysters (*Crassostrea gigas*) subjected to ultraviolet-irradiated seawater. Applied Microbiology, 23 (1): 11-16.
- Vaughn, J. M. and Metcalf, T. G., 1975. Coliphages as indicators of enteric viruses in shellfish and shellfish raising estuarine waters. Water Research, 9: 613-616.
- Vaughn, J. M., Landry, E. F., Thomas, M. Z., Vicale, T. J. and Penello, W. F., 1979. Survey of human Enterovirus occurrence in fresh and marine surface waters on Long Island. Applied and Environmental Microbiology, 38 (2): 290-296.

- West, P. A., 1991. An Overview of the Bivalve Molluscan Shellfish Industry and Depuration Practices in the United Kingdom. In: W. S. Otwell, G. E. Rodrick and R. E. Martin (eds). *Molluscan Shellfish Depuration*, CRC Press, Boca Raton, FL, pp. 275-285.
- Wohlgeschaffen, G. D., Mann, K. H., Scubba Rao, D. V., and Pocklington, R. Dynamics of the phycotoxin domoic acid: accumulation and excretion in two commercially important bivalves. *Journal of Applied Phycology*, 4: 297-310.
- Wood, P. C. and Ayres, P. A., 1977. *Artificial Seawater for Shellfish Tanks*. Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, Lowenstoft, U.K., 11 pp.
- Wright, A. C., Miceli, G. A., Landry, W. L., Christy, J. B., Watkins, W. D. and Morris, J. G., Jr., 1993. Rapid identification of *Vibrio vulnificus* on nonselective media with an alkaline phosphatase-labelled oligonucleotide probe. *Applied and Environmental Microbiology*, 59 (2): 541-546.
- Wyatt, G.M., Lee, H.A., and Morgan, M.R.A. (eds), 1992. *Immunoassays for Food Poisoning Bacteria and Bacterial Toxins*. Chapman and Hall, London, U.K, 129 pp.
- Yoovidhya, T. and Fleet, G. H., 1981. An evaluation of the A-1 Most Probable Number and the Anderson and Baird-Parker plate count methods for enumerating *Escherichia coli* in the Sydney rock oyster (*Crassostrea commercialis*). *Journal of Applied Bacteriology*, 50: 519-528.

## **Appendix 1**

Dr Chris Burke and Dr Greg Maguire procured much of the literature cited in this review and prepared an initial draft of the document on behalf of the NSW Shellfish Quality Assurance Program.

Peer review of the original draft was undertaken by Dr Peter Ayres and Professor Graham Fleet, and their constructive comments have been incorporated in the final report.

Dr Kerry Jackson, Dr Timothy Shaw, Mr Damian Ogburn and Mr Andrew Derwent contributed to editing of the final draft of the document.

The assistance of Ms Jane Frances in the preparation of tables and references, and Mr Sam Lynch in document formatting is gratefully acknowledged.

**Other titles in this series:****ISSN 1440-3544**

- No. 1 Andrew, N.L., Graham, K.J., Hodgson, K.E. and Gordon, G.N.G., 1998. Changes after 20 years in relative abundance and size composition of commercial fishes caught during fishery independent surveys on SEF trawl grounds. Final Report to Fisheries Research and Development Corporation. Project no. 96/139.
- No. 2 Virgona, J.L., Deguara, K.L., Sullings, D.J., Halliday, I. and Kelly, K., 1998. Assessment of the stocks of sea mullet in New South Wales and Queensland waters. Final Report to Fisheries Research and Development Corporation. Project no. 94/024.
- No. 3 Stewart, J., Ferrell, D.J. and Andrew, N.L., 1998. Ageing Yellowtail (*Trachurus novaezelandiae*) and Blue Mackerel (*Scomber australasicus*) in New South Wales. Final Report to Fisheries Research and Development Corporation. Project no. 95/151.
- No.4 Pethebridge, R., Lugg, A. and Harris, J., 1998. Obstructions to fish passage in New South Wales South Coast streams. Final report to Cooperative Research Centre for Freshwater Ecology.
- No. 5 Kennelly, S.J. and Broadhurst, M.K., 1998. Development of by-catch reducing prawn-trawls and fishing practices in NSW's prawn-trawl fisheries (and incorporating an assessment of the effect of increasing mesh size in fish trawl gear). Final Report to Fisheries Research and Development Corporation. Project no. 93/180.
- No. 6 Allan, G.L., and Rowland, S.J., 1998. Fish meal replacement in aquaculture feeds for silver perch. Final Report to Fisheries Research and Development Corporation. Project no. 93/120-03.
- No. 7 Allan, G.L., 1998. Fish meal replacement in aquaculture feeds: subprogram administration. Final Report to Fisheries Research and Development Corporation. Project no. 93/120.
- No. 8 Heasman, M.P., O'Connor, W.A., O'Connor, S.J., 1998. Enhancement and farming of scallops in NSW using hatchery produced seedstock. Final Report to Fisheries Research and Development Corporation. Project no. 94/083.
- No. 9 Nell, J.A., McMahon, G.A., and Hand, R.E., 1998. Tetraploidy induction in Sydney rock oysters. Final Report to Cooperative Research Centre for Aquaculture. Project no. D.4.2.
- No. 10 Nell, J.A. and Maguire, G.B., 1998. Commercialisation of triploid Sydney rock and Pacific oysters. Part 1: Sydney rock oysters. Final Report to Fisheries Research and Development Corporation. Project no. 93/151.

- No. 11 Watford, F.A. and Williams, R.J., 1998. Inventory of estuarine vegetation in Botany Bay, with special reference to changes in the distribution of seagrass. Final Report to Fishcare Australia. Project no. 97/003741.
- No. 12 Andrew, N.L., Worthington D.G., Brett, P.A. and Bentley N., 1998. Interactions between the abalone fishery and sea urchins in New South Wales. Final Report to Fisheries Research and Development Corporation. Project no. 93/102.
- No. 13 Jackson, K.L. and Ogburn, D.M., 1999. Review of depuration and its role in shellfish quality assurance. Final Report to Fisheries Research and Development Corporation. Project no. 96/355.