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Australian Marine Biotoxin Management Plan for Shellfish Farming

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Australian Marine Biotoxin Management Plan for Shellfish Farming

Prepared for

Australian Shellfish Quality Assurance Advisory Committee (ASQAAC)

by

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AUTHOR'S NOTE

During the preparation of this report, the EC DSP expert working group notified new draft legislation (SANCO/2227/2001 Rev 3) for the DSP group of toxins, and is summarised as follows:

Article 1 - The decision sets down the detection methods and regulatory limits for Diarrhetic Shellfish Poisoning (DSP) complex toxins (Okadaic Acid and Dinophysistoxins), Yessotoxins, Pectenotoxins and Azaspiracids. It applies to bivalve molluscs, echinoderms, tunicates and marine gastropods that are intended for immediate human consumption or for further processing before consumption.

Article 2 - The regulatory limit for **total content of Okadaic Acid, Dinophysistoxins and Pectenotoxins** in the whole body or any part edible separately of those animals in Article 1, and intended for human consumption is fixed at **16 µg/100 g**. The methods of analysis are referred to below.

Article 3 - The regulatory limit for **Yessotoxins** in the whole body or any part edible separately of those animals in Article 1, and intended for human consumption is fixed at **100 µg of Yessotoxin Equivalents/100 g**. The methods of analysis are referred to below.

Article 4 - The regulatory limit for **Azaspiracids** in the whole body or any part edible separately of those animals in Article 1, and intended for human consumption is fixed at **16 µg of Azaspiracid Equivalents/100 g**. The methods of analysis are referred to below.

Article 5 - When there are discrepancies demonstrated between results of analytical methods, then the mouse bioassay should be considered the reference method.

Detection Methods
Biological methods
- A mouse bioassay with acetone extraction can be used to detect Okadaic acid, Dinophysistoxins, Pectenotoxins and Yessotoxins. This assay may be complemented if necessary with liquid/liquid partition steps with ethyl/acetate/water or dichloromethane/water to remove potential interferences. Azaspiracid detection at the regulatory levels by means of this procedure requires the use of the whole body as the test portion.
- A mouse bioassay with acetone extraction followed by liquid/liquid partition with diethyl ether can be used to detect Okadaic Acid, Dinophysistoxins and Pectenotoxins but it cannot be used to detect Yessotoxins and Azaspiracids as losses of these toxins may take place during the partition step.
- The rat bioassay can detect Okadaic Acid, Dinophysistoxins and Azaspiracids.

Alternative detection methods
A series of methods such as High Performance Liquid Chromatography (HPLC) with fluorimetric detection, Liquid Chromatography (LC)-Mass Spectrometry (MS), immunoassays and functional assays such as the phosphatase inhibition assay, can be used as alternative of complementary methods to the biological testing methods, providing that either alone or combined they can detect the following analogues:
- Okadaic acid and Dinophysistoxins: a hydrolysis step may be required in order to detect the presence of DTX3;
- Pectenotoxins: PTX1 and PTX2;
- Yessotoxins: YTX, 45 OH YTX, Homo YTX and 45 OH Homo YTX;
- Azaspiracids: AZA1, AZA2 and AZA3.

EXECUTIVE SUMMARY

In Australia, as in many countries, aquaculture and wild harvest of shellfish is an economically important and growing industry. The safety of these products as a food source is of utmost importance from both public health and economic points of view. One of the potential problems faced by shellfish growers is the contamination of their product with marine biotoxins. These are chemical compounds (toxins) that are produced by specific naturally occurring marine microalgae. Most microalgae (a.k.a. phytoplankton) are actually an important food source of the shellfish. These biotoxins can induce human illness if contaminated shellfish are consumed. This is not only a problem for commercially produced or harvested shellfish; it is also a problem for recreational shellfish gatherers, for some of which this may be subsistence gathering.

Biotoxins are not only a problem for Australia, as most coastal countries in the world have had, or have the potential for, problems with marine biotoxin contamination in shellfish. In order to manage this problem, many countries have monitoring programs aimed at the detection of the species of microalgae that produce the toxins, and for the toxins themselves in the shellfish. Monitoring for the microalgae is a faster and cheaper test than shellfish testing, and may give an early warning of the potential for contamination of shellfish with marine biotoxins. However, the two types of testing need to be performed in conjunction with each other. Internationally, food safety regulations are based on the levels of toxins in shellfish, and it is these results that should generally be used for regulatory decisions. It is a common misconception that cooking or processing the shellfish in some way will remove the toxins and make the shellfish safe to eat, in some instances the toxin compounds can be converted into more toxic compounds by cooking.

Internationally the impacts of toxic microalgae on both public health and the economy are increasing in frequency, intensity and geographic distribution. As aquaculture expands, and its importance as both food and income sources increases for many countries, it is expected that these impacts will also increase. As international markets become more conscious of the safety of the foodstuffs they import, they impose safety regulations and can impose non-trade barriers.

Australia's shellfish industry's market has a large domestic component, with shellfish landings worth approximately $90M per year. There is, perhaps, less external pressure on Australia to manage these problems. However the domestic market is large, and the consumers no less important than overseas consumers, and hence there remains the need for protection from marine biotoxins. There need to be controls in place between states, just as there need to be controls for exporting product. The USA has a similar political structure to Australia, with both state and national governments, and in order to protect the public health of shellfish consumers in other states, a model ordinance was implemented which all states must ratify to ensure meeting the standards set out in this document. This document is a voluntary agreement between states, and spells out the acceptable monitoring programs, controls and regulations that must be met in order to 'export' shellfish to another signatory state. This model ordinance is fairly well accepted as an international standard for shellfish safety, along with the European Union directives, which must be met in order to export shellfish to the EU.

This report summarises the available information on:

- State marine biotoxin monitoring programs for cultured shellfish,
- Internationally recognised management practices,
- Methodologies for marine biotoxin analysis,
- The risk of marine biotoxins to public health,
- The microalgae posing the risk and their temporal and regional occurrences,
- The industries that are at risk, and

- The food safety controls and regulatory mechanisms.

This report is in two part: Part A - A Review of Marine Biotoxin Management in Australia; and Part B - A Model Australian Marine Biotoxin Management Plan.

Currently Australia has no national guidance on marine biotoxin monitoring, although there are programs conducted in most states to varying degrees. One of the difficulties in implementation of a national strategy has been the lack of reliable information on and knowledge about the history of the occurrence of toxic microalgae and marine biotoxins in some shellfish growing areas. This project has involved a review of the monitoring programs and the history of potentially toxic microalgae for all the states. Victoria, Tasmania, South Australia and New South Wales have already experienced closures or human illness due to marine biotoxins. New Zealand has detected all temperate biotoxin producing microalgal genera, and has also found most of the tropical genera in the sub-tropical northern regions. The Australian coastline encompasses all climate zones and it is expected that all biotoxin producing species will be detected over time, and that they will bloom as conditions become favourable to them.

There is currently a lack of consistency in marine biotoxin management between the states, which must be addressed. For a national marine biotoxin strategy to succeed there needs to be commitment from all states to participate in and meet the requirements of the program.

One of the key aspects in successful monitoring programs is having ongoing research underpinning the program. There needs to be more investigation of the microalgae species that are present in Australian waters, including culturing them and testing for toxin production. It is only after this work is undertaken that action levels relevant to Australia can be set. In the meantime, action levels are based on international experience, and may not necessarily fit the Australian situation. Other important research that will strengthen monitoring programs is the investigation of the uptake, retention and biotransformation of toxins in shellfish; some species take up toxins more quickly, some depurate toxins more quickly, and some bio-transform toxins into different (and potentially more toxic) compounds. This research is on going internationally, and as more research is done, more questions are asked. Federal funding (eg Fisheries Research Development Corporation or Australia Research Council) is required for many of these research questions.

The funding of a monitoring program, however, is not the responsibility of such agencies. The costs of programs need to be shared by all users, which enhances the coverage of monitoring information, and reduces the direct cost for the industry. Internationally, shellfish safety tends to be managed by either Health or Fisheries Departments, however in Australia, the situation varies between states. There needs to be commitment and support from both State and Federal governments, and in particular between fisheries and health agencies, but not excluding Environmental Protection Agencies, Sewage Authorities, Port Authorities, Aboriginal Commissions, and other stakeholders. Countries such as Canada, USA and New Zealand invest approximately 1-2% of the value of the industry in biotoxin monitoring. Currently Australia invests approximately 0.02% in biotoxin monitoring.

There needs to be the open sharing of data between all players in the monitoring, and this includes researchers. If there is a sharing of cost, then there also tends to be a sharing of information. One of the positive outcomes of this is that research can become targeted towards the real issues that the shellfish industry faces. In order to achieve this goal of openness, there need to be clear channels of communication, and roles and responsibilities clearly delineated. There also needs to be on going education of the industry, regulators and policy makers.

A marine biotoxin monitoring program is a long-term commitment to protecting the public health of shellfish consumers, understanding more about the shellfish resource and assisting the industry to growing into the future. It requires regulatory commitment at Federal and State government level to maintain and police biotoxin standards.

RECOMMENDATIONS

- A Marine Biotoxin Monitoring Program (a model forms Part B of this report) is accepted and implemented nationally, and is included in the ASQAAC Program Managers manual.

- AQIS audits of the Shellfish Quality Assurance Program in each State or Territory include auditing the marine biotoxin program against the Marine Biotoxin Monitoring Program.

Administration

- An 'Australian National Biotoxin Program', a co-operative program requiring the support and commitment of the Federal Government and State and Northern Territory Governments, should be established either within or in close association with the Australian Shellfish Quality Assurance Program (ASQAP).

- The Ministerial Council on Agriculture, Forestry and Fisheries – Australia (AFFA), acting through the Standing Committee on Fisheries and Aquaculture (SCFA) (or the appropriate Standing Committee), should accept responsibility for the governance of the marine biotoxin issue.

- SCFA (or the other appropriate Standing Committee) should be strongly represented on the 'Natural Toxins Working Group' of the Standing Committee on Agriculture and Resource Management (SCARM), which would benefit the seafood industry by becoming more involved with the well-organised beef and grain industries.

- The Australian Shellfish Quality Assurance Advisory Committee (ASQAAC) should report directly to SCFA (or the other appropriate Standing Committee), not via a sub-committee of SCFA, to raise the profile of biotoxin management within Australia.

- ASQAAC membership should include representatives of commercial wild harvest shellfish industries (e.g. scallops and pipis).

- Biotoxin management sections (including Appendix VI 'Suggested Contingency Plan for Control of Marine Biotoxins') of the 'Operations Manual of the Australian Shellfish Sanitation Control Program' should be substantially revised and updated, especially to ensure that routine micro-algal monitoring *and* appropriate flesh testing is conducted.

- Agreements or Memoranda of Understanding concerning the interstate trading of shellfish, similar to that contained in the U.S. 'Model Ordinance' should be developed between the States and Territories. All States and Territories would then need to satisfy agreed standards in order to sell shellfish interstate.

- A National database of all microalgal, biotoxin, and related environmental data, and case history investigations, should be further investigated. This could be maintained by AFFA, and be funded by Federal Government.

Funding

- Sufficient and equitable funding should be provided by relevant State Government agencies (acting for "public good") and by a levy on shellfish industries to implement an adequate biotoxin monitoring program in all shellfish harvesting areas.

- The roles and responsibilities of all Government agencies and shellfish industries should be clearly defined in each State to determine the basis for equitable funding contributions. A Premier's Department or State Cabinet directive may be required to achieve the active participation of all relevant State Government agencies in addition to the primary or lead agency.

- Other interested parties such as Environmental Protection Agencies, Water and Sewage Authorities, Port Authorities, Aboriginal Commissions and other relevant organisations should offer support and information sharing in future routine and contingency monitoring programs.

- Appropriate contingency funding should be available in each State to enable microalgal and biotoxin monitoring to be rapidly expanded in the event of a large toxic algal bloom.

Communication

- Clear and open communication networks should be established both at National and State levels and written into management plans.

- A central State database (possible web based) must be established and maintained to store all the phytoplankton monitoring, biotoxin, related environmental data and suspected toxic shellfish poisoning case investigations.

- There must be clear definition of roles and responsibilities of all Federal and State agencies involved in marine biotoxin monitoring.

Management Plans

- For those States and Territory that do not have a plan in place, a clear and comprehensive 'Marine Biotoxin Management Plan', which meets the needs of the State and is consistent with the requirements of the Australian National Biotoxin Program, must be implemented.

- For those growing areas in each State and territory that do not have a plan in place, a 'Marine Biotoxin Management Plan' relevant to that growing area, that includes routine (sentinel) monitoring and a contingency plan, must be implemented.

- All State monitoring programs and growing area management plans should be kept up to date and reviewed annually to ensure the plans are effective and reflect current operating procedures. All management plans should be audited annually as part of the SQAP AQIS audits.

- Pectenotoxins and yessotoxins should continue to be classified as DSP toxins, which have a regulatory limit of 20 µg okadaic acid equivalent/100 g as specified in the 'Australia New Zealand Food Standards Code'. Internationally, there is a lack of epidemiological evidence on the human health effects of these toxins and their associated dose response characteristics. Draft EC guidelines have been released which set levels of 16 µg/100 g total content of okadaic acid, dinophysistoxins and pectenotoxins; and 100 µg yessotoxin equiv./100 g (See Author's note page iv).

- Marine biotoxin controls for commercial wild harvest shellfish must be developed and included in the ASQAP requirements.

- Phytoplankton monitoring should be conducted weekly to be the most effective. This is the internationally accepted frequency, and should be increased when necessary due to blooms.

- Risk assessments should be undertaken for areas with no history of toxic algal blooms or biotoxins in shellfish. These assessments should involve weekly phytoplankton monitoring and shellfish monitoring for biotoxins and could involve sediment surveys for toxic algal cysts.

- In new areas, or in areas with little historic information, shellfish samples should be taken regularly (weekly or fortnightly) in association with water samples to collect data and increase knowledge of the area.

- Monitoring programs should include both routine phytoplankton monitoring and shellfish flesh testing. Regulatory decisions concerning the closure or re-opening of a shellfish growing area should be made based on flesh results. The phytoplankton data should be used to trigger further sampling and toxin testing.

- Regular and routine phytoplankton and biotoxin monitoring should be conducted to provide continuous public health protection.

- Biotoxin safety limits documented in marine biotoxin management plans should conform to the regulatory limits specified in the 'Australia New Zealand Food Standards Code'. The Australia New Zealand Food Authority (ANZFA) standards are recognised internationally as having appropriate safety margins.

- All State and Territory management plans should include closure and re-opening criteria for all marine biotoxins. Both sets of criteria, and guidelines for their application, should conform to the relevant requirements of the Australian National Biotoxin Program.

- During a biotoxin event, as much information as possible should be collected, and should include phytoplankton and biotoxin monitoring data (including results from additional sampling), environmental data, and investigation reports on suspected poisoning cases.

- All States and Territories should use a standard case investigation form for the investigation of suspected clinical cases of shellfish poisoning. A thorough investigation based on sound epidemiological principles should be followed in every case.

- Case investigation reports should be stored in a central State database along with phytoplankton, biotoxin and any other data pertinent to the investigation.

- The Northern Territory should develop a marine biotoxin management plan and a contingency plan to guide urgent management action in the event a biotoxin event should occur.

- NSW and Queensland should urgently implement routine phytoplankton monitoring and shellfish flesh testing in those growing / harvest areas where it is not already in place.

- Biotoxin monitoring programs should have industry support, scientific input and direction from State Government. Local shellfish industry members should be encouraged to play an active role in the implementation of all monitoring programs.

- Monitoring programs should be implemented for wild harvest shellfish industries and harvest areas not currently monitored in all States.

Education

- Education and understanding of marine biotoxins is vitally important for all participants in a marine biotoxin monitoring program from industry personnel to program mangers to regulators and policy makers.

- A regular meeting such as a workshop is a good forum for education. All parties should be invited such as research scientists, laboratory staff, program managers, industry personnel, regulators and other interested parties. People should be encouraged to give short presentations about their current work and issues of concern to them.

- Public education should be ongoing, in order to minimise the 'halo' effect of publicity of shellfish safety during marine biotoxin events.

Laboratories

- An approval system for both laboratories and methods, in order to perform testing for the Monitoring Program is implemented for both phytoplankton and biotoxin laboratories. At a minimum this should be NATA accreditation, with additional market access requirements, e.g. USFDA, as necessary.

- Laboratories need to be able to offer expert advice, and have directly relevant training (e.g. attendance at UNESCO courses).

- Proficiency testing programs should be set up especially for the laboratory personnel involved in identification of marine microalgae.

- Biotoxin laboratories need to participate in national and international inter-laboratory calibration programmes.

- There need to be more laboratories with a greater emphasis on marine phytoplankton. Laboratories need to clearly differentiate between NATA accreditation for freshwater and marine analysis.

Phytoplankton

- Phytoplankton analysis needs to target all potentially toxic species.

- Many programs place a lot of emphasis on qualitative net tow sampling, which may fail to detect certain toxic species. Quantitative sampling methods such as bottle and hose sampling need to be implemented more widely.

- Sampling methods used should be standardised by all states, and training workshops should be held regularly to ensure accurate and consistent sampling.

Biotoxin

- Laboratories need to be able to analyse for all biotoxins, ensuring that all States have access to testing capability.

- Test results need to be available as soon as possible and freighting delays need to be minimised, in order for a management plan to work effectively.

- Management plans should incorporate testing for all biotoxins.

Research

- A National research strategy should be put in place to avoid overlapping of research effort. Research priorities need to be established and funding needs to be made available to undertake the priority projects. This funding should come from Commonwealth agencies.

- Toxicity testing of cultured phytoplankton species needs to be undertaken for many species in all States.

- During marine biotoxin events, additional species of shellfish should be collected and tested to gain information about toxin uptake, retention times

ABBREVIATIONS

AFFA	Agriculture Forestry and Fisheries Australia
ANZFA	Australian New Zealand Food Authority
AOAC	Association of Official Agricultural Chemists
APEC	Asia Pacific Economic Co-operation
APHA	American Public Health Association
AQIS	Australian Quarantine and Inspection Services
ARC	Australia Research Council
ARNAT	Australian Research Network for Algal Toxins
ASP	Amnesic Shellfish Poisoning
ASQAAC	Australian Shellfish Quality Assurance Advisory Committee
ASQAAP	Australian Shellfish Quality Assurance Advisory Program
ASQAP	Australian Shellfish Quality Assurance Program
AZA	Azaspiracid
AZP	Azaspiracid Shellfish Poisoning
BTX	Brevetoxin
CFIA	Canadian Food Inspection Agency
CFP	Ciguatera Fish Poisoning
CRIMP	Centre for Research on Introduced Marine Pests
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DA	Domoic Acid
DFO	Department of Fisheries and Oceans
DHHS	Department of Health and Human Services
DHWA	Department of Health Western Australia (HDWA)
DLWC	Department of Land and Water Conservation
DNRE	Department of National Resources and Environment
DPIWE	Department of Primary Industries ,Water and Environment
DSF	Division of Sea Fisheries
DSP	Diarrhetic Shellfish Poisoning
DTX	Dinophysis toxin
EEC	European Economic Community
ELISA	Enzyme Linked Immunosorbent Assay
EPA	Environment Protection Authority
EU	European Union
FDA	Food and Drug Administration
FRDC	Fisheries Research Development Council
FRST	Foundation for Research Science and Technology
FWA	Fisheries Western Australia
GBRMPA	Great Barrier Reef Marine Park Authority
HAB	Harmful Algal Bloom
HPLC	High Performance Liquid Chromatography
HPLC/FD	High Performance Liquid Chromatography with Fluorescence detection
HPLC/UV/DAD	High Performance Liquid Chromatography with Ultra Violet single wavelength or Diode Array Detection
ICES	International Council for the Exploration of the Sea
IOC	Inter Governmental Oceanographic Commission
IPA	Intertidal Protected Areas
ISO/IEC	International Standards Organisation/
LC-MS	High Performance Liquid Chromatography with Mass Spectrometry detection

MAFRI	Marine and Freshwater Resources Institute
MOU	Memorandum Of Understanding
MU	Mouse Units
NATA	National Association of Testing Authorities
NMR	Nuclear Magnetic Resonance
NSSP	National Shellfish Sanitation Program
NPF	Northern Prawn Fishing
NSP	Neurotoxic Shellfish Poisoning
NSWSQAP	New South Wales Shellfish Quality Assurance Program
NZMAF/MAF	New Zealand Ministry of Agriculture and Forestry
OA	Okadaic Acid
PIRSA	Primary Industry Resource South Australia
PP2A	Protein Phosphatase Inhibition Assay
PSP	Paralytic Shellfish Poisoning
PTX	Pectenotoxin
PTX2sa	Pectenotoxin 2 seco acid
QDEH	Queensland Department of Environmental Heritage
QDOD	Queensland Department of Health
QDPI	Queensland Department of Primary Industry
QOGA	Queensland Oyster Growers Association
QSWAMP	Queensland Shellfish Water Assurance Monitoring Program
QX	(disease)
RACC	Regional Algal Co-ordination Committee
RIS	Respiratory Irritation Syndrome
SACC	State Algal Co-ordinating Committee
SADHS	South Australian Department of Human Services
SASQAP	South Australian Shellfish Quality Assurance Program
SAX	Strong Anion Exchange
SCARM	Standing Committee on Agriculture and Resource Management
SCFA	Standing Committee on Fisheries and Aquaculture
SPE	Solid Phase Extraction
SSCA	State Shellfish Control Agency
STX	Saxitoxin
SW	Sydney Water
TSP	Toxic Shellfish Poisoning
TSQAP	Tasmanian Shellfish Quality Assurance Program
UNESCO	United Nations Education, Scientific and Cultural Organisation
USFDA	United States Food and Drug Administration
UV	Ultra Violet
VSQAP	Victorian Shellfish Quality Assurance Program
WASQAP	Western Australian Shellfish Quality Assurance Program
WES	Water ECOscience
WHO	World Health Organisation
WRC	Water and Rivers Commission
YTX	Yessotoxin

PART A

Australian Marine Biotoxin Management Plan for Shellfish Farming

A review of current national and international practices

PART A – A REVIEW OF NATIONAL AND INTERNATIONAL PRACTICES

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1 INTRODUCTION

1.1 Background

The commercial culture and wild harvest of shellfish species for human consumption is an economically viable business in many countries including Australia. The non-commercial harvest of wild sh40ellfish provides a food source for recreational shellfish gatherers and may, in some areas, be an important subsistence food source. The safety of shellfish is therefore the focus of many monitoring and research programs around the world, especially in instances where they are exported to international markets.

Bivalve shellfish (such as mussels, scallops, oysters and clams) filter large volumes of seawater from which they remove particles such as microalgae (also known as phytoplankton). There are approximately 5000 species of marine microalgae, of which approximately 40 or so species produce marine biotoxins (Hallegraeff 1995). These toxins are chemical compounds that can induce human illness if contaminated shellfish are consumed. When the environmental conditions are suitable, the microalgae can rapidly divide and multiply in number to the point where the water becomes discoloured, forming what is known as an algal 'bloom', 'red-tide' or 'harmful algal bloom (HAB)'. Not only toxin producing species form 'blooms' and the majority of microalgae species are a good food source for the shellfish. While bivalve shellfish are the most important vectors of concern, marine biotoxins have also been found in carnivorous and scavenging gastropods and crustaceans (Arnott 1998). Biotoxins are also produced by some species of freshwater 'blue-green' algae (also known as cyanobacteria). Some toxin-producing marine microalgae only need to be present at very low cell concentrations in order for shellfish to accumulate toxins above regulatory levels, in which case, there does not need to be a visible 'bloom' in order for the shellfish to be toxic.

There are four main shellfish poisoning syndromes, each of which has more than one causative toxin responsible for it. These are Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP) and Neurotoxic Shellfish Poisoning (NSP). There is also a recognised illness caused by aerosol affects of cells breaking up in surf (Respiratory Irritation Syndrome (RIS)). In addition, there is Ciguatera Fish Poisoning (CFP) which is caused by eating certain species of tropical reef fish. The causative microalgae live in epiphytic association with particular seaweed species, in the sediment and in coral. The toxins are passed through the food chain from small grazing fish into the organs of the larger predatory fish (Hallegraeff 1995). The symptoms of shellfish poisoning range from gastrointestinal and neurological symptoms, to death in the worst cases.

Shellfish naturally depurate the toxins if they are left in the water, although some shellfish species take longer to depurate than others, and once harvested the toxins cannot be removed. Contaminated shellfish usually look and taste the same as uncontaminated product. Biotoxins cannot be removed from shellfish by cooking or rendered harmless by cooking or marinating, and in fact these processes can convert some toxins into more toxic compounds.

The international impacts of toxic microalgae on both public health and the economy, are increasing in frequency, intensity and geographic distribution (Hallegraeff 1995). With the increasing importance of aquaculture as a food and income source for many countries, it is expected that these impacts will continue to multiply. As international markets become more conscious of the safety of the foodstuffs they import, they impose safety regulations and can impose non-trade barriers. For countries such as New Zealand, where a large proportion of their aquaculture product is exported, these regulatory measures exert pressure on the industry to meet these standards. Shellfish products also need to meet internal food safety standards to promote the image of safe seafood products.

Many countries manage the problem of marine biotoxins by using monitoring programs based on a combination of phytoplankton monitoring[1] and testing of the shellfish flesh itself. Phytoplankton monitoring acts as an early warning of potential marine biotoxin problems in shellfish, the testing is quicker and more cost-effective than monitoring the shellfish for the toxins, and it reduces the unnecessary disposal of harvested contaminated product (Todd 1999). These monitoring programs need to be well planned, with relevant legislative backing, and designated authorities with the necessary approval to take action as required. They need to have appropriate and sufficient funding, which also enables contingency testing in the case of a marine biotoxin event. The program and the laboratories performing analyses need to be audited regularly.

This study has been undertaken to provide Australia with a National Marine Biotoxin Strategy. Many states currently have, or in the past have had, marine biotoxin monitoring programs, but there is no uniformity across the nation. Similar studies have been conducted, such as the 1993 National Residue Survey report by Hallegraeff and Sumner. However this did not lead to the implementation of an Australian-wide monitoring strategy. The main aim of this project has been the implementation of such a program. This has involved a comprehensive review of current monitoring programs, the history and occurrence of toxic and potentially toxic marine microalgae around Australia, an assessment of the risk marine biotoxins pose to public health, a review of monitoring programs used internationally, and summarises the Food Safety Regulations. We have also developed a Model National Marine Biotoxin Management Plan (Cawthron Report No. 646).

1.2 Scope of Project

The following objectives and methods were supplied as a guideline for the project content.

Objective

To design a biotoxin monitoring strategy, in consultation with government and industry, that will encompass each Australian State and the Northern Territory, and which will:

- *identify those organisms that pose a biotoxin threat to marine and estuarine shellfish in Australian waters and identify those industries at risk;*
- *review existing monitoring programs and analytical expertise, and identify deficiencies;*
- *identify gaps in current methodology for the identification and measurement of toxins;*
- *assess the risk to public health posed by marine biotoxins;*
- *identify internationally recognised practises for the management of biotoxins in shellfish; and*
- *determine a suitable process by which data can be consolidated, collated and analysed to assist in the public health protection of shellfish consumers.*

The strategy will also be required to provide:

- data to underpin food safety controls and regulatory mechanisms;
- data to help identify regional and temporal occurrences of hazardous levels of biotoxins in shellfish;
- a model system for early warning of potential shellfish biotoxin problems to commercial and recreational shellfish sectors; and
- a model protocol for resuming safe shellfish harvesting after biotoxin contamination has closed a shellfish harvesting area.

[1] Phytoplankton monitoring is the term commonly used to describe monitoring of water column samples for microalgal species which are potential marine biotoxin producers. Some species such as *Prorocentrum lima, Ostreopsis* spp. and *Coolia monotis* are more commonly benthic or epiphytic, but are also seen in the water column. Phytoplankton monitoring is sometimes also called HAB monitoring.

Method 1

Identify those organisms that pose a biotoxin threat to marine and estuarine shellfish in Australian waters and identify those industries at risk.

To achieve this objective it will be necessary, at a minimum, to:

- review the literature for recorded toxic algae blooms in Australia;
- review the results from monitoring programs currently undertaken in Australia; and
- assess the risks from introductions of biotoxin-producing organisms from overseas.

Method 2

Review existing monitoring programs and analytical expertise, and determine possible deficiencies and opportunities for improvements.

To achieve this objective it will be necessary:

- for State Shellfish Control Authorities to provide the consultant with summaries of State biotoxin monitoring programs;
- to evaluate the level of infrastructure and expertise available for relevant analytical services; and
- to establish what other measures may be in place within Australia for biotoxin monitoring and control.

Method 3

Identify gaps in current methodology for the identification and measurement of biotoxins.

The consultant will need to:

- review the literature for the methods used to measure the various biotoxins;
- assess the relative specificity and sensitivity of methodologies for known biotoxins; and
- assess those incidences in which it has been impossible to identify the source of the biotoxin.

Method 4

Assess the risk to public health posed by marine biotoxins.

The consultant should conduct an assessment of circumstances and conditions in which shellfish consumer safety is threatened (by biotoxin contamination) through evaluation of those measures and programs currently in place and the gaps and deficiencies in monitoring and control practices prevailing in the commercial shellfish sector.

Method 5

Identify internationally recognised practises for the management of biotoxins in shellfish

This will require a literature review of international models for the management of marine biotoxins in shellfish.

Method 6

To determine a suitable process for the consolidation, collation and analysis of data on biotoxins to assist in the public health protection of shellfish consumers.

The consultant will need to:

- liaise with appropriate organisations and agencies involved in the management of biotoxins to assess the potential for maximising the exchange of data and information between them;
- determine thorough consultation whether there is a need for collation of data within a central agency and what this data could be used for;
- determine who would benefit from a formal system of data collation and data analysis;
- determine a strategy for co-ordinating data processing and the development of predictive and management tools; and
- explore possibilities for relevant funding.

1.3 Focus of This Report

This report is focused at the commercial harvesting of bivalve shellfish whether cultured or wild harvest, although there is inevitably some overlap with the recreational gathering of shellfish. The State reviews undertaken primarily deal with cultured bivalve shellfish, however many of the programmes in place are equally applicable to both commercial and non-commercial wild harvest of shellfish. Shellfish such as abalone are not covered in this report. Part B of this report is a model plan for marine biotoxin monitoring, this is equally applicable to both culture and wild harvest of shellfish This report deals with PSP, DSP, ASP and NSP; it does not address issues related to CFP and freshwater cyanobacteria and the biotoxins they produce.

1.4 Shellfish Poisoning Descriptions

1.4.1 Paralytic Shellfish Poisoning (PSP)

- **Causative toxins:** Saxitoxins (STXs), Gonyautoxins (GTXs) and C toxins (CTXs)
- **Microalgal sources:** *Gymnodinium catenatum*, *Alexandrium* species (including *A. minutum*, *A. catenella, A. tamarense, A. fundyense, A. ostenfeldii*, plus others), *Pyrodinium bahamense* var. *compressum*, also freshwater species such as *Anabaena* spp., and *Microcystis* spp.
- **Associated Health Hazards:** This group of toxins affects the nervous system by causing blockage of the sodium channels. In humans the peripheral nervous system is particularly affected; symptoms include tingling and numbness of extremities, progressing to lack of muscular co-ordination, respiratory distress, and muscular paralysis leading to death by asphyxiation in extreme cases. The fatality rate can be up to 10%. There is no known antidote.
- **Clinical Case Definition:** The following neurological symptoms occurring within 12 hours of consuming shellfish:
  - neurosensory;
  - paraesthesia, i.e. numbness or tingling around the mouth, face or extremities;
  - and one of the following neuromotor/neurocerebellar symptoms:
    - weakness such as trouble rising from seat or bed
    - difficulty in swallowing
    - difficulty in breathing
    - paralysis
    - clumsiness
    - unsteady walking
    - dizziness/vertigo
    - slurred/unclear speech
    - double vision

1.4.2 Amnesic Shellfish Poisoning (ASP)

- **Causative toxins:** Domoic acid (DA)
- **Microalgal sources:** *Pseudo-nitzschia* species including *P. australis, P. multiseries, P. delicatissima, P. fraudulenta, P. pseudodelicatissima* plus others.
- **Associated Health Hazards:** Domoic acid affects the brain. A mild case of ASP causes nausea, vomiting, diarrhoea and abdominal cramps within 3-5 hours of consumption. Severe cases have a decreased reaction to deep pain, dizziness, hallucinations, confusion, short-term memory loss and seizures. The most severe cases have been found to have selective memory loss, particularly short-term memory loss. There appears to be a close association between memory loss and age: those people under 40 years old are more likely to have diarrhoea and those over 50 to have memory loss.

- **Clinical Case Definition:**
  - Vomiting or diarrhoea or abdominal cramps within 24 hours of consuming shellfish;
  - and no other probable cause identified by microbiological examination of a faecal specimen from the case or microbiological testing of left-over food;
  - and/or one or more of the following neurological signs/symptoms occurring within 48 hours of consuming shellfish:
    - confusion
    - memory loss
    - disorientation
    - seizure
    - coma

1.4.3 Diarrhetic Shellfish Poisoning (DSP)

- **Causative toxins:** Okadaic acid (OA), Dinophysistoxins (DTXs), Pectenotoxins (PTXs), Yessotoxins (YTXs) and Azaspiracids (AZAs). NB. The human toxicity of pectenotoxins and yessotoxins is currently unknown, until proven non-toxic to humans they will continue to be regulated for as DSP toxins. Azaspiracids are not yet confirmed to be in this group.
- **Microalgal sources:** *Dinophysis* species including *D. acuminata, D. acuta, D. caudata, D. fortii, D. norvegica* plus others, *Prorocentrum lima, Protoceratium reticulatum* (YTX)
- **Associated Health Hazards:** Okadaic acid and the dinophysistoxins cause diarrhoea, vomiting, nausea and abdominal pain. The symptoms usually start between 30 minutes to a few hours after consumption. There is concern that okadaic acid and dinophysistoxins also cause longer term health effects. These possible human health affects have been associated with tumour producing, mutagenic and immunosuppressive effects shown in animals. These human health concerns have yet to be epidemiologically qualified and quantified.
  There has been some debate as to whether pectenotoxins cause human health effects. However, there has now been a documented illness outbreak in New South Wales that involved pipis. Fifty-six persons became ill with vomiting and diarrhoea and the pipis were found to contain PTX2sa (Quilliam *et al.* 2000). Another 50 cases were thought to be involved in a similar NSW outbreak, associated with recreational harvest of pipis.
  There is no epidemiological evidence of human health effects from yessotoxin. However it is lethal to mice when administered intraperitoneally, and causes damage to heart muscles and livers in mice.
  Azaspiracids cause vomiting and diarrhoea in humans. In animal tests, these toxins have caused neurotoxic effects and severe damage to the intestine, spleen and liver tissues. The microalgal source is currently unconfirmed.
- **Clinical Case Definition:**
  - Vomiting or diarrhoea occurring within 24 hours of consuming shellfish;
  - and no other probable cause identified by microbiological examination of a faecal specimen from the case or microbiological testing of leftover food.

1.4.4 Neurotoxic Shellfish Poisoning (NSP)

- **Causative toxins:** Brevetoxins (BTX's)

- **Microalgal sources:** *Karenia brevis* (=*Gymnodinium breve*), *K.* cf *brevis* (=*Gymnodinium* cf *breve*), plus potentially *K. papilionacea* (=*Gymnodinium papilionaceum), K. mikimotoi* (=*Gymnodinium mikimotoi*) and similar species; *Chattonella* species, *Heterosigma akashiwo* and *Fibrocapsa japonica*.

- **Associated Health Hazards:** The symptoms occur within 3-5 hours and are chills, headache, diarrhoea, muscle weakness, joint pain, nausea and vomiting. There can be altered perceptions between hot and cold, difficulty in breathing, double vision, trouble in walking and swallowing.
- **Clinical Case Definition:** Two or more of the following neurological symptoms occurring within 24 hours of consuming shellfish:
  - neurosensory:
    - paraesthesia, i.e. numbness or tingling around the mouth, face or extremities
    - alternation of temperature sensations such as a prickly feeling on the skin during a bath/shower or exposure to sun, or difficulty distinguishing hot or cold objects
  - neuromotor/neurocerebellar:
    - weakness such as trouble rising from seat or bed
    - difficulty in swallowing
    - difficulty in breathing
    - paralysis
    - clumsiness
    - unsteady walking
    - dizziness/vertigo
    - slurred/unclear speech
    - double vision

1.5 ANZFA Food Standards

The Australian New Zealand Food Authority (ANZFA) develops standards and associated draft codes of practice and guidelines. ANZFA also has a role in co-ordinating monitoring and surveillance activities in relation to food, and in developing food education initiatives to increase public awareness. Currently the Australian Food Code lists the following standards for bivalve molluscs.

The edible portion of bivalve molluscs

i) Must not contain a level of PSP greater than 0.8mg/kg when determined by the method of the A.O.A.C., 15th Edition (1990), Section 959.08:

ii) Must not contain a level of domoic acid greater then 20 mg/kg when determined by the A.O.A.C., 15th Edition (1990), 2nd Supplement (1991), Section 991.26.

The New Zealand standards list regulatory levels for four toxin groups. Therefore ANZFA have recommended that the Joint Australia New Zealand Food Standards Code incorporate standards for all four toxins. The regulatory levels proposed are:

PSP: Equal or greater than 80 µg/100 grams in edible part of shellfish.
ASP: 20 ppm of domoic acid in edible part of shellfish
DSP: Equal or greater than 20 µg/100 grams in edible part of shellfish
NSP: Equal or greater than 20 MU/100 grams in edible part of shellfish

2 IMPLEMENTATION OF A NATIONAL STRATEGY

2.1 Why Does Australia Need Marine Biotoxin Monitoring and Management?

The ultimate aim of marine biotoxin and phytoplankton monitoring is to prevent human illnesses (and in extreme cases death) after seafood consumption. In doing so, biotoxin monitoring provides a quality assurance for the seafood industry, which in turn creates consumer confidence in Australian seafood and indirectly confidence in Australia's comparatively unpolluted marine environment. A biotoxin monitoring strategy such as this, attracts important economic benefits by creating market opportunities for domestic as well as international seafood markets (e.g. through APEC), and indirectly also for marine tourism activities.

Even if the commercial shellfish industries take all possible precautions, an outbreak of shellfish poisoning resulting from recreational shellfish gathering still has the potential to cause considerable economic damage. This is due to the unpredictable nature of harmful algal blooms, and the speed with which toxin levels can accumulate in shellfish.

Routine biotoxin monitoring is essential in all shellfish growing areas, whether they are commercially cultured, commercially wild harvested or recreational gathered shellfish.

2.2 Cost Benefits

While the risk of human fatalities after seafood consumption is significantly smaller than the risk of, for example, a fatal car accident, it is a widely held social decision that food products should be free from harmful contaminants. The Australian beef and grain export industries have, at times, suffered significant economic losses (and loss of consumer confidence) after contamination scares and are now very well organised to deal with natural toxins.

By contrast the Australian seafood industry is leaving itself dangerously exposed. Countries such as Canada, the USA and Europe invest 1-2% of the value of their seafood industries in biotoxin monitoring. The Australian seafood industry valued at $1.8 B /yr (of which aquaculture contributes $600M and shellfish alone $90M) currently does not invest more than $350,000/yr (0.02%) in phytoplankton and marine biotoxin monitoring. For a country with a population of 18.3 million people, which takes pride in its large coastline, this represents a serious neglect of a highly valued public amenity. By comparison, New Zealand spends $3.2 M/yr in monitoring to protect a seafood export industry valued at NZ $1.43B (of which $314M is shellfish), that is, the monitoring effort costs approximately 2% of the value of the industry.

2.3 Existing Regulatory Approaches

Impediments to Australia's development of an effective biotoxin monitoring strategy have been:

(i) The lack of leadership at the Commonwealth Government level as to whether Departments of Health, Environment or Fisheries should take governance of the algal biotoxin issue. Internationally, either Health or Fisheries Departments tend to assume control for seafood safety issues. Examples of the first approach are the US Food and Drug Administration or the Japanese Ministry of Health, while an example of the latter approach is the New Zealand Ministry of Agriculture and Forestry (MAF). A global overview of management structures for biotoxins can be found at http://ioc.unesco.org/hab/data2.htm#1.

(ii) Australian States do not tolerate Federal interference in their internal affairs, and any national program therefore must be a cooperative one.

(iii) The lack of consistency between Australian States, which exhibit varying commitment and regulations relating to the biotoxin problem. Historically the unwillingness by New South

Wales, as the largest Australian oyster producer (valued at $30M/yr), to take part in ASQAP until 1999 was a major stumbling block towards a National Marine Biotoxin Strategy. Shellfish from NSW are not eligible for export to certain markets due to non-compliance with the ASQAP. A significant shellfish poisoning outbreak in NSW has, however, the potential to adversely affect the reputation of the entire Australian seafood industry (the so-called "halo-effect" of bad publicity).

(iv) Previous coverage of the biotoxin issue (since 1988) by the Australian Quarantine and Inspection Service (AQIS), through its Australian Shellfish Quality Assurance Advisory Committee (ASQAAC, initially named Australian Shellfish Sanitation Advisory Committee, ASSAC) has been limited to a jurisdiction of seafood export certification. The Australian Shellfish Quality Assurance Program (ASQAP) is coordinated by ASQAAC which includes industry and State and Commonwealth government representatives and reports to a subcommittee of the Standing Committee on Fisheries and Aquaculture.

(v) The Australian focus on fisheries production for domestic markets rather than overseas export markets (Australian shellfish landings are valued at $90M/yr, of which exports contribute $40M/yr). The opposite situation applies to the New Zealand seafood industry, where 90% of the mussel production (valued at NZ$170 M/yr) is exported.

2.4 Suggested Improvements in Australia's Biotoxin Management Structure

(i) The Ministerial Council on Agriculture, Forestry and Fisheries -Australia (AFFA), through the Standing Committee on Fisheries and Aquaculture (SCFA), appears to be the obvious candidate to take on governance of the marine biotoxin issue. This should also include strong SCFA representation on the natural toxins working group of the Standing Committee on Agriculture and Resource Management (SCARM). This allows the seafood industry to benefit from the better organised beef and grain industries.

(ii) The ASQAAC profile needs to be raised, with direct reporting to SCFA necessary.

(iii) A more prominent role for the National Residue Survey needs to be explored.

(iv) Wild harvest shellfisheries (especially pipis, scallops), currently are not covered by ASQAAP, and need to be integrated into the National Marine Biotoxin Monitoring Program.

(v) The 1997 ASQAP "Manual of Operations" for the Shellfish Industry needs to be substantially updated and rewritten, to include phytoplankton and marine biotoxin monitoring, in addition to the existing contingency plans for marine biotoxin events. State programs need to be regularly audited, with compulsory reporting requirements.

(vi) While there is wide recognition of the value of phytoplankton and marine biotoxin monitoring, high level direction is needed from State Premiers Departments or from the individual State cabinets, before responsible agencies can sort out their mutual roles and responsibilities and associated funding structures.

(vii) The highly successful New Zealand concept of six monthly Marine Biotoxin workshops is worthy of introduction into Australia. By inviting industry, regulators, health officers, fisheries officers, laboratory personnel, funding agencies etc., issues can be discussed with input from all interested parties. This promotes an atmosphere of openness and prevents duplication of efforts.

3 INTERNATIONALLY RECOGNISED PRACTICES FOR THE MANAGEMENT OF MARINE BIOTOXINS IN SHELLFISH

3.1 International Shellfish Quality Assurance Models

Internationally there are two key models that marine biotoxin monitoring programs are based on and designed to meet. These are the United States of America Food and Drug Administration (USFDA) National Shellfish Sanitation Program (NSSP) Model Ordinance and the European Union (EU) Directive 91/492/EEC. The NSSP Model Ordinance is a guidance document which States agree to accept in order to market shellfish to other states, and generally adopt this into their legislation. The Directive 91/492/EEC is enforced by regulation and must be met in order for any country to export shellfish to the European Union. New draft legislation by the EC, which alters the levels for the DSP group of toxins, has been notified in SANCO/2227/2001 Rev 3. The USFDA also have a suggested contingency plan for control of Marine Biotoxins. This has been designed following shellfish poisoning outbreaks in areas previously unaffected by marine biotoxin and demonstrates the importance of being prepared in the event of an outbreak.

3.1.1 USFDA

United States Food and Drug Administration, National Shellfish Sanitation Program (NSSP) Model Ordinance 1999

This document "represents the Agency's current thinking on the safe and sanitary control of the growing, processing, and shipping of molluscan shellfish for human consumption." In relation to marine biotoxin monitoring in Chapter IV. Shellstock Growing Areas it states that:

"04 Marine Biotoxin Control.

A. Contingency Plan.

(1) The Authority shall develop and adopt a marine biotoxin contingency plan for all marine and estuarine shellfish growing areas.

(2) The plan shall define the administrative procedures and resources necessary to accomplish the following:

(a) Initiate an emergency shellfish sampling and assay program;

(b) Close growing areas and embargo shellfish;

(c) Prevent harvesting of contaminated species;

(d) Provide for product recall;

(e) Disseminate information on the occurrences of toxic algal blooms and/ or toxicity in shellfish meats to adjacent states, shellfish industry, and local health agencies; and

(f) Coordinate control actions taken by Authorities and federal agencies.

(3) Except that the Authority shall classify as prohibited any growing areas where shellfish are so highly or frequently affected by marine biotoxins that the situation cannot be safety managed, the presence of marine biotoxins shall not affect the classification of the shellfish growing area under §.03. The Authority may use the conditionally approved classification for areas affected by marine biotoxins.

(4) The plan may include agreements or memoranda of understanding, between the Authority and individual shellfish harvesters, to allow harvesting in designated parts of a growing area while other parts of the growing area are placed in the closed status. Such controlled harvesting shall be conducted with strict assurances of safety, such as by batch release of shellfish lots only after samples of each lot are tested and found to be below the action levels specified in §C.

B. Marine Biotoxin Monitoring. In those areas where marine biotoxins are likely to occur in shellfish, representative samples of shellfish shall be collected during all harvest periods. Samples shall be collected from indicator stations at intervals determined by the Authority, and assayed for the presence of toxins in accordance with §C.

C. Closed Status of Growing Areas.

(1) A growing area, or portion(s) thereof as provided in §A.(4), shall be placed in the closed status for the taking of shellstock when the Authority determines that the level of biotoxin present in shellfish meats is sufficient to cause a health risk. The closed status shall be established based on the following criteria:

(a) The concentration of paralytic shellfish poison (PSP) equals or exceeds 80 micrograms per 100 grams of edible portion of raw shellfish; or

(b) For neurotoxic shellfish poisoning (NSP), the harvesting of shellstock shall not be allowed when:

(i) Any NSP toxin is found in shellfish meats; or

(ii) The cell counts for Gymnodinium breve organisms in the water column exceed 5,000 per liter; or

(c) For domoic acid, the toxin concentration shall not be equal to or exceed 20 ppm in the edible portion of raw shellfish.

(2) For any marine biotoxin producing organism for which criteria have not been established under this Ordinance, either cell counts in the water column or biotoxin meat concentrations may be used by the Authority as the criteria for not allowing the harvest of shellstock.

(3) When sufficient data exist to establish that certain shellfish species can be safely exempted from the marine biotoxin contingency plan, the closed status for harvesting may be applied selectively to some shellfish species and not others.

(4) The closed status shall remain in effect until the Authority has data to show that the toxin content of the shellfish in the growing area is below the level established for closing the area.

(5) The determination to return a growing area to the open status shall consider whether toxin levels in the shellfish from adjacent areas are declining.

(6) The analysis upon which a decision to return a growing area to the open status is based shall be adequately documented.

D. Heat Processing. If heat processing is practiced, a control procedure shall be developed. This procedure shall define the following:

(1) Toxicity limits for processing;

(2) Controls for harvesting and transporting the shellstock to processor;

(3) Special marking for unprocessed shellstock;

(4) Scheduled processes; and

(5) End product controls on the processed shellfish.

E. Records. The Authority shall maintain a copy of all of the following records:

(1) All information, including monitoring data, relating to the levels of marine biotoxins in the shellfish growing areas;

(2) Copies of notices placing growing areas in the closed status;

(3) Evaluation reports; and

(4) Copies of notices returning growing areas to the open status. "

3.1.2 European Union Directive 91/492

Council Directive 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve molluscs (includes amendment Council Directive 97/61/EC of 20 October 1997)

In relation to marine biotoxin monitoring in Chapter VI Public Health Control and Monitoring of Production , it states:

"A public health control system must be established by the competent authority in order to verify whether the requirements laid down in this Directive are complied with. This control system must include:

1. periodic monitoring of live bivalve mollusc relaying and production areas in order to:…

> *…(c) check the possible presence of toxin-producing plankton in production and relaying waters and biotoxins in live bivalve molluscs;…*

…For the purposes of points (c) and (d), sampling plans must be established by the competent authorities for checking such possible presence at regular intervals or on a case-by-case basis in the event of irregular periods of harvesting.

2. Sampling plans as provided for in point 1, must in particular take account of:…

> *…(b) possible variations in production at relaying areas in the presence of plankton containing marine biotoxins. The sampling must be carried out as follows:*
>
> *(i) monitoring: periodic sampling organized to detect changes in the composition of the plankton containing toxins and the geographical distribution thereof. Information leading to a suspicion of accumulation of toxins in mollusc flesh must be followed by intensive sampling;*
> *(ii) intensive sampling:*
> - *monitoring plankton in the growing and fishing waters by increasing the number of sampling points and the number of samples, and*
> - *toxicity tests using the molluscs from the affected area which are most susceptible to contamination.*
>
> *Placing on the market of molluscs from that area may not be re-authorized until new sampling has provided satisfactory toxicity test results;*

(c) possible contamination of the molluscs in the production and relaying area;

If the result of a sampling plan shows that placing on the market of live bivalve molluscs may constitute a hazard to human health, the competent authority must close the production area, as regards molluscs concerned, until the situation has been restored.

3. Laboratory tests in order to check compliance with the requirements for the end product as laid down in Chapter V of this Annex. A control system must be established to verify that the level of marine biotoxins does not exceed safety limits…."

In relation to marine biotoxin regulatory levels in Chapter V Requirements Concerning Live Bivalve Molluscs, it states:

"6. The total Paralytic Shellfish Poison (PSP) content in the edible parts of molluscs (the whole body or any part edible separately) must not exceed 80 micrograms per 100 g of mollusc flesh in accordance with the biological testing method – in association if necessary with a chemical method for detection of Saxitoxin – or any other method recognized in accordance with the procedure laid down in Article 12 of this Directive.

If the results are challenged, the reference method shall be the biological method.

7. The customary biological testing methods must not give a positive result to the presence of Diarrhetic Shellfish Poison (DSP) in the edible parts of molluscs (the whole body or any part edible separately).

7a. The total Amnesic Shellfish Poison (ASP) content in the edible parts of the molluscs (the entire body or any part edible separately) must not exceed 20 micrograms of domoic acid per gramme using the HPLC method."

3.1.3 EEC Draft Legislation notified in SANCO/2227/2001 Rev 3

This decision sets out the detection methods and regulatory limits for the DSP complex (Okadaic acid and Dinophysistoxins), Yessotoxins, Pectenotoxins and Azaspiracids, and applies to bivalve molluscs, echinoderms, tunicates and marine gastropods intended for human consumption either immediately or following further processing (Article 1).

"Article 2
The regulatory limit for total content of Okadaic Acid, Dinophysistoxins and Pectenotoxins in the animals referred to in Article 1 (the whole body or any part edible separately) and intended for human consumption, is fixed at 1 6µg/100 g. The methods of analysis are defined in the Annex to this Decision."

"Article 3
The regulatory limit for Yessotoxins in the animals referred to in Article 1 (the whole body or any part edible separately) and intended for human consumption, is fixed at 100 µg of Yessotoxin Equivalent/100 g."

"Article 4
The regulatory limit for Azaspiracids in the animals referred to in Article 1 (the whole body or any part edible separately) and intended for human consumption, is fixed at 16 µg of Azaspiracid equivalents/100 g."

3.1.4 USFDA Contingency Plan

A.2 – Suggested Contingency plan for Control of Marine Biotoxins (USFDA NSSP, Guidance documents 1999)

"II Recommended Program Guidelines

A. Provide early warning system:

1. *Procedures should be established with natural resource agencies to report to marine biotoxin control officials any abnormal environmental phenomenon that might be associated with shellfish growing areas, such as bird or fish kills, water discoloration, and abnormal behaviour or shellfish or marine scavengers.*

2. *An early warning, shellfish monitoring program should be implemented. The monitoring program should include the "key station" and "critical species" concepts. Frequency of sampling should adequately monitor fluctuations in coastal phytoplankton populations.*

3. *Channels of communication concerning fluctuations in shellfish toxicity should be established with other states, MOU countries, FDA, and other responsible officials. A marine biotoxin control official should be designated by the state shellfish control agency to receive and distribute all marine biotoxin related information.*

B. *Define severity of problem:*

1. *A procedure should be established to promptly expand the sampling program for marine biotoxins in the event of increased toxicity at any key monitoring stations within the state, MOU country, or in adjacent states. Such a procedure should include plans for obtaining the additional resources necessary to implement the expanded sampling and laboratory analysis program. Information should be available concerning the location of commercial shellfish resource areas in the state.*

2. *Criteria should be developed to define under what circumstances areas will be closed to harvesting because of marine biotoxin contamination. The criteria should integrate public health, conservation, and economic considerations. Principal items of concern include number of samples required to initiate action, size of area to be closed (including a safety zone), and type of harvesting restrictions to invoke (all species or specific species).*

3. *Procedures should be established to promptly identify which shellfish products or lots might potentially be contaminated, and to determine the distribution of these products or lots.*

C. *Respond effectively to minimize illness:*

1. *A summary should be provided citing the laws and regulations in states or MOU countries which promptly and effectively allow the state shellfish control agency to restrict harvesting, withdraw interstate shipping permits, and to embargo any potentially toxic shellfish already on the market in the event of a marine biotoxin episode. Special consideration should be given to defining the time frame involved in taking appropriate legal action.*

2. *The administrative procedures necessary to close areas, withdraw interstate certification, and to embargo shellfish should be defined. The time frame necessary to accomplish these actions should also be defined.*

3. *A plan should be developed which will define what type of patrol program will be necessary to properly control harvesting in contaminated areas, and test this program to assure prompt implementation in the future.*

4. *Procedures should e developed to disseminate information on the occurrences of toxic algal blooms to the industry and local health agencies.*

5. *Procedures should be established to co-ordinate control actions taken by state and federal agencies or departments and district, regional, or local health authorities.*

D. Gather follow-up data:

1. Appropriate records of illnesses should be complied and maintained by the SSCA. These should include data on the incidence of illness and appropriate case history data. This information may be important in defining the severity of the problem, as well as for a retrospective evaluation of the adequacy of the entire control program.

2. Records of toxic shellfish sample results should include analyses of trends, detoxification curves, phytoplankton and water sample analyses, and pertinent environmental observations.

E. Reclaim harvesting areas

1. Once an area is closed because of marine biotoxin contamination, a procedure should be instituted to gather data necessary to decide when the area can be reopened. A system of representative samples to establish detoxification curves can be part of this procedure.

2. The SSCA should develop a set of criteria which must be met before an area can be reopened. This criteria should integrate public health, conservation, and economic considerations.

3. *A program of consumer education should be continued as long as any area remains contaminated and closed."*

3.2 Types of Marine Biotoxin Monitoring Programs Used Internationally

Internationally marine biotoxin monitoring is undertaken by employing the use of phytoplankton monitoring, shellfish flesh monitoring, or a most commonly, a combination of both systems. Monitoring may employ highly trained laboratory experts, or may use volunteer networks. Monitoring may be for cultured shellfish species, or for a combination of cultured and wild harvest species. Monitoring programs are generally either government funded or funded by industry.

The information quoted here is taken from Andersen's 1996 review of harmful marine microalgae monitoring which was based on a questionnaire compiled by the ICES-IOC Working Group on Harmful Algal Bloom Dynamics. Forty-four questionnaires were returned of which 30 had phytoplankton monitoring programs in place. With the inclusion of information from other sources, there were a total of 43 countries/regions known to have phytoplankton monitoring programs in place.

3.2.1 Phytoplankton Monitoring Only

Phytoplankton monitoring is rarely used on it's own as a complete monitoring program, and is not considered acceptable on it's own by either the European Union (EU) or the United States Food and Drug Administration (USFDA). Where it forms part of an integrated management program, phytoplankton monitoring information can initiate management action in 30% of countries/regions.

3.2.2 Phytoplankton /Flesh Combination

This is the most commonly followed program for marine biotoxin management internationally, with 70% of Harmful Algal Bloom (HAB) monitoring programs initiated for the management of molluscs either from cultured stock or wild harvest, 55% of programs used for finfish culture. Of

these programs, 64% quantify toxins in molluscs, and in some countries (Canada, Italy, Portugal, Spain-Galicia, USA-California and Venezuela) toxins are also quantified in fish.

Management actions can be initiated based on quantification of algae in 30% of the countries/regions, 47% initiate management actions based on algal toxins, and 57% initiate management actions based on both sets of results. In Denmark and New Zealand closures can be initiated based on concentrations of harmful algae.

3.2.3 Government Operated

Eighty two percent of the management programs have been initiated and planned by government authorities. Four countries (Canada (West Coast), Chile, Denmark and Norway) have programs initiated by private organisations. Denmark, Italy, Netherlands, Norway, Spain (Valencia) and USA (state of Washington) have programs initiated and planned by a combination of government and private organisations.

Government food control authorities are responsible for the management actions based on the data in 60% of the countries/regions. Public health authorities undertake the action in 61% of cases. In 24% of the cases, pollution control authorities used the data.

Government agencies fund the monitoring programs in 91% of cases. In Denmark and Chile, fisheries associations finance monitoring. In New Zealand government funding finances the monitoring for the recreational program. In Norway, Netherlands, parts of the USA, and Canada private users pay for the monitoring data. Research institutions pay for the monitoring programs in Finland, Norway and Portugal.

3.2.4 Industry Operated

Aquaculturalists and fishermen use the data for management purposes in 64% of the cases. In Denmark and Chile, fisheries associations finance monitoring. In New Zealand the commercial program is financed by industry. In New Zealand and Denmark, the industry fishermen play a major role in the monitoring program in that they carry out sampling for both algae and shellfish. They then freight the samples to private laboratories for analysis.

3.2.5 Volunteer Systems

In several states in the USA, volunteer networks are used for monitoring phytoplankton. For example in the State of Maine, they are primarily monitoring for PSP in shellfish. The phytoplankton monitoring program is volunteer based, with community members and students using 20 µm plankton nets and field microscopes to identify *Alexandrium* spp., *Dinophysis* spp., *Prorocentrum lima* and *Pseudo-nitzschia* spp. This type of sampling provides qualitative results only. This acts as an early warning, and primary, secondary and tertiary flesh monitoring sites are used when they need to test for biotoxins. Shellfish are also collected state-wide from April to October and tested for PSP.

3.3 Examples of Programs

3.3.1 New Zealand

The New Zealand shellfish industry is based on both wild harvest and culture of shellfish species. New Zealand put in place an extensive marine biotoxin monitoring program in 1993 following an NSP outbreak which resulted in the entire country being closed for shellfish harvesting (Trusewich 1996). Since 1993 the program has evolved dramatically, and is now two programs - one for the commercial shellfish industry and one for the recreational shellfish gathering areas. The main aims

of the commercial program are to prevent harvest of contaminated product and to protect the public health of consumers of shellfish whether commercially or recreationally harvested. The main aim of the recreational program is the protection of public health.

The Ministry of Agriculture and Forestry (MAF) administers the industry program, while the Ministry of Health (MoH) administers the recreational program. However there is some overlap between the two as Health Protection Officers (employed by Health Authorities) play a major role in the running of industry programs in their areas. Quantitative water samples are collected weekly, and analysed for phytoplankton. Results of these tests are faxed to the client sending the sample, as well as to the local health representative, and in the case of industry samples, the regional shellfish specialist (MAF representative). Samples of shellfish are also collected at the same time, or at set intervals (weekly, fortnightly or monthly) depending on the area management plan. Shellfish may be tested for different toxins at different intervals depending on the management plan, for example, a site might be tested for PSP every week, DSP and ASP every fortnight, and NSP monthly. If at any time a trigger level (Table 1) of a particular phytoplankton species is met, then a sample must be tested for the toxin relating to that species.

Table 1. Trigger levels for phytoplankton used in New Zealand.

Phytoplankton species	Toxin	Level in composite sample to trigger flesh testing (cells per litre)	Industry voluntary closure pending flesh testing results (cells per litre)	Issue public health warning (cells per litre)
Alexandrium minutum	PSP	100	500	5000
Alexandrium ostenfeldii	PSP	100	500	5000
Alexandrium catenella	PSP	100	500	5000
Alexandrium tamarense	PSP	100	500	5000
Gymnodinium catenatum	PSP	100		
Pseudo-nitzschia spp (>50% total phytoplankton)	ASP	50 000	200 000	N/A
Pseudo-nitzschia spp (<50% total phytoplankton)	ASP	100 000	500 000	N/A
Gymnodinium cf breve	NSP	1000	5000	5000
Dinophysis acuta	DSP	500	1000	N/A
Dinophysis acuminata	DSP	1000	2000	N/A
Prorocentrum lima	DSP	500	1000	N/A

For *Alexandrium* species and *Gymnodinium catenatum*, the trigger level is set at the detection level of the method, so that when these species are detected in a sample, flesh testing must be undertaken. When a toxin test is positive, but below the regulatory flesh limit (Table 2), extra sampling of phytoplankton and shellfish may be undertaken. When a shellfish test gives a positive result above the regulatory limit, the area is closed and testing continues until the area can be re-opened according to the re-opening criteria (Table 2).

Table 2. Regulatory shellfish toxin levels for closing and re-opening areas in New Zealand.

Toxin	Method	Close	Re-open
PSP	Mouse bioassay	>= 80 ug/100 g STX equivalent	< 80 ug/100 g in two consecutive samples over a minimum of 14 days.
DSP	DSP/NSP Screen – acetone extraction Mouse Bioassay Confirmatory tests	>= 20 ug/100 g	<20 ug/100 g in two consecutive samples taken not less than 7 days apart.
NSP	DSP/NSP Screen – acetone extraction Mouse Bioassay Confirmatory – ether extraction mouse bioassay	20 MU/100 g	<20 MU/100 g in two consecutive samples of which the second must be taken no earlier than 2 days after the initial clear sample.
ASP	HPLC	20 ppm	<20 ppm in three consecutive samples over a minimum of 14 days.
Lipid soluble toxins (eg yessotoxin and others e.g. DTX3)	DSP/NSP Screen – acetone extraction Mouse Bioassay	If DSP/NSP Screen is positive, confirmatory tests are negative and causative phytoplankton are present, then may close.	

3.3.2 Denmark

The Danish mussel industry is based on wild harvest of primarily blue mussels (*Mytilus edulis*) (Emsholm *et al.* 1996). The monitoring program has been in place since 1991 with the primary goals being:

1) consumer protection from toxic mussels; and
2) optimising fishing effort by harvesting from low risk areas.

The program was initiated following the occurrence of DSP-toxins in exported mussels. The program uses weekly sampling of both phytoplankton and toxins in shellfish. Denmark monitors for DSP, PSP and ASP.

The coastline has been divided into grids or areas, and the status of the grids (whether open, open with restrictions or closed) is kept up to date on an automatic telephone answering machine, which the fisherman and industries can ring. To begin fishing in a grid, the fishermen collect samples of mussels as well as both qualitative (20 µm plankton net) and quantitative (water sampler) water samples the week before harvesting is due to start. When harvesting starts, each boat collects phytoplankton and mussel samples to be analysed on the first fishing day every week. Results are sent to the Danish Fish Inspection Service, Ministry of Fisheries, individual mussel industries and the secretariat of the Danish Association of Musselfisheries. The Fish Inspection Service decides whether the areas are open, closed or under intensified monitoring.

Table 3. Trigger levels for phytoplankton used in Denmark.

Phytoplankton species	Level for closed/intensified monitoring (cells per litre)
Dinophysis acuminata	500
Dinophysis acuta	500
Dinophysis norvegica	10^3
Dinophysis rotundata	10^3
Total *Dinophysis* spp.	1.2×10^3
Prorocentrum lima	500
Alexandrium ostenfeldii	500
Alexandrium tamarense	500
Alexandrium spp.	500
Pseudo-nitzschia seriata-group	$2x10^5$
Pseudo-nitzschia delicatissima-group	$5x10^5$
Pseudo-nitzschia spp.	$2x10^5$
Nodularia spumigena	$1\text{-}2x10^5$

Table 4. Regulatory shellfish toxin levels for closing areas in Denmark.

Toxin	Method	Close
DSP	Mouse bioassay: – ether extraction (official method); – acetone extraction (used for *Mytilus edulis* under normal surveillance). Verification by HPLC	DSP toxins detected
PSP	Mouse bioassay verification by HPLC	>= 80 µg/100 g
ASP	HPLC	>=2 mg/100 g

3.3.3 Canada

The Canadian shellfish industry is based on both wild harvest and shellfish culture. The main purposes of the monitoring program are public health protection and product safety for domestic and export markets.

Canada monitors for PSP, ASP and DSP and the Canadian Food Inspection Agency (CFIA) is the agency responsible for collecting and analysis of shellfish samples. The Department of Fisheries and Oceans (DFO) implement and enforce closures based on recommendations made by CFIA.

Some areas use phytoplankton e.g. the Bay of Fundy which has water samples collected weekly from May through October, biweekly during November and May, and monthly during December through April (Andersen 1996).

The causative organisms of PSP are *Alexandrium fundyense* and *A. tamarense* on the Atlantic Coast, and *A. tamarense* and *A. catenella* on the Pacific Coast. The causative organisms of ASP are *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima.*

Table 5. Trigger levels for phytoplankton used in Canada.

Phytoplankton species	Critical concentration (cells/litre)	Regulation
Alexandrium fundyense	Presence in the water	Measure toxins in shellfish
Pseudo-nitzschia multiseries	>50,000	Measure toxins in shellfish
Pseudo-nitzschia pseudodelicatissima	>50,000	Measure toxins in shellfish

Table 6. Regulatory shellfish toxin levels for closing and re-opening areas in Canada.

Toxin	Method	Close	Re-open
			Within a period of 14 days, a minimum of three samples have been sampled and found to contain:
PSP	Mouse bioassay	>=80 µg/100 g	<= 80 µg/100 g
ASP	HPLC	>=20 µg/g	<= 20 µg/
DSP	Mouse bioassay (HPLC/ELISA)	OA and/or DTX1 singly or in combination >=1 µg/g (equiv approx to 20 µg/100 g soft tissue	OA and/or DTX1 singly or in combination <=1 µg/g (equiv approx to 20 µg/100 g soft tissue

Exceptions: 1) Soft shell clams and mussels (Atlantic) may be harvested when PSP toxin levels exceed 80 µg/100g and are less than 160 µg/100 g.

2) Butter clams on the West Coast may be harvested and canned, subject to the following condition, when the PSP toxin levels (µg/100 g) are:

>300 to <= 500 entire siphon must be removed
> 80 to <= 300 distal half of the siphon must be removed
<= 80 black tip of the siphon must be removed.

3.4 Discussion

These models are designed to ensure the safety of shellfish exported either to the USA or EU. The USFDA model ordinance was originally designed to ensure safety of shellfish between states, and could be used in a similar manner within Australia as a means of ensuring the food safety of shellfish on the domestic market also.

The basic requirements for any marine biotoxin monitoring program established in any country are as follows:

(i) There should be a planned program that can be adapted to suit different areas on a case by case basis, without altering the main aims of the program. A planned program has scope (for example the monitoring of both recreational and commercial areas, both cultured and wild harvest shellfish), it spells out the agencies involved, the type of monitoring is noted and the frequency of this monitoring. The sites that are monitored are recorded. There is a contingency plan for increasing sampling as necessary, and this includes an increase in the capacity of laboratories to analyse samples. Notification procedures for results are documented, as are procedures for closure and re-opening of areas. There are documented procedures for the recall and detention of contaminated shellfish and/or for public warnings in the case of recalls or area closures. There are also surveillance procedures for closed areas to ensure harvesting product isn't continuing.

(ii) The program has clear and relevant legislative backing available (on both a state and national level), and this legislation is concise and ensures authorities can take the appropriate action.

(iii) There is appropriate and sufficient funding available to carry out the monitoring program. There is also sufficient contingency funding available for use in the case of a marine biotoxin event.

(iv) Internationally the best practice is a combination of phytoplankton monitoring and flesh testing. This is determined on a case by case basis, and monitors for known and potential

risks in an area. In a country like Australia with a coastline that encompasses all climate zones, it is likely that all known marine biotoxin producing species will be detected over time, and they will bloom as conditions become favourable to them.

(v) Phytoplankton monitoring plays an important role as an early warning, however internationally it is principally flesh testing that area closures are based on with a few exceptions that use phytoplankton. In all cases, shellfish testing should be carried out in conjunction with phytoplankton monitoring.

(vi) Phytoplankton action levels and closure levels need to be established based on the information relevant to that area.

4 METHODOLOGIES FOR MARINE BIOTOXIN ASSAY AND ANALYSIS

4.1 Introduction

A literature review of the methods used to measure levels of the various marine biotoxin groups in shellfish is presented and the relative specificity, sensitivity and overall efficacy of these methods are evaluated. Where possible, comments have been made on those incidents in Australia and New Zealand in which it has been impossible to identify the source of the biotoxin. This review comes at a time when over a decade of international research has developed a variety of new analytical and assay procedures that could play a role in improving the accuracy, speed and cost effectiveness of marine biotoxin surveillance programs. A number of alternative tests have good performance characteristics, however, few have yet been through the full validation and certification procedures which are necessary for acceptance by regulatory authorities and therefore the mouse bioassay remains the only internationally accepted method for most toxins. At present the best alternative methods closest to validation that can deliver high levels of accuracy, sensitivity and rapidity for monitoring are the immuno-assay test kits and chemical chromatography with mass spectrometry detection (e.g. High Performance Liquid Chromatography with Mass Spectrometry detection (LC-MS)). The mouse bioassay remains the main officially sanctioned method of screening for most toxin groups in Australia and New Zealand (Table 7), however its drawbacks are widely acknowledged and over the last decade there has been a considerable international research effort to develop alternative methods.

Table 7. Summary of action levels and current assay and analysis methods for marine bio-toxins

Toxin group	Regulatory action level	Current accepted regulatory method	Possible future regulatory methods	Other methods
PSP-toxins	20 µg STX equivalents/100 g	Mouse bioassay	ELISA (eg. Mist Alert™) LC-MS	Neuroblastoma assay HPLC-FD Receptor binding assay Saxiphilin assay
ASP-toxins	20 mg/100 g	HPLC-UV/DA HPLC-FD	ELISA (eg. Mist Alert™) LC-MS	Mouse bioassay
OA & DTXs*	20 µg/100 g	Mouse bioassay ELISA (DSP Check)	HPLC-FD LC-MS	PP2A assay
NSP-toxins	20 MU/100 g	Mouse bioassay	LC-MS	HPLC-FD Neuroblastoma assay Receptor binding assay ELISA
YTXs*	20 µg/100 g	Mouse bioassay	LC-MS HPLC-FD	ELISA
PTXs*	20 µg/100 g	Mouse bioassay	LC-MS HPLC-FD	

HPLC-UV/DA = High performance liquid chromatography with ultraviolet single wavelength or diode-array detection
HPLC-FD = High performance liquid chromatography with fluorescence detection
LC-MS = High performance liquid chromatography with mass spectrometry detection
PP2A = Protein phosphatase inhibition assay
MU = Mouse Units
* = Internationally these toxin groups are classified as DSP toxins, and it is expected they will remain so until toxicology work is undertaken to prove otherwise, allowing the setting of separate regulatory levels.

The mouse bioassay for water and lipid soluble toxin groups is expensive and cumbersome and generally has a low level of sensitivity and specificity, but especially for the latter (Table 8). In

addition there is the potential for resistance from national and international consumers opposed to the use of animals for laboratory testing.

Table 8. Relative specificity and sensitivity of available methods of marine biotoxin analysis

Toxin group	Test method	Specificity	Sensitivity
PSP-toxins	Mouse bioassays	Moderate	Low
	ELISA (e.g. Mist Alert™)	High	High
	HPLC-FD	High	High
	LC-MS	Very High	High
	Neuroblastoma assay	Moderate	Moderate
	Receptor binding assay	Moderate	Moderate
	Saxiphilin assay	High	High
Domoic acid (ASP-toxins)	Mouse bioassay	Low	Low
	HPLC-UV/DA	Moderate	High
	HPLC-FD	High	High
	ELISA (e.g. Mist Alert™)	High	High
	LC-MS	Very High	Very High
Okadaic acid and Dinophysistoxins (DSP-toxins)	Mouse bioassay	Low	Low
	ELISA (e.g. DSP Check ™)	Moderate	Moderate
	HPLC-FD	High	High
	LC-MS	Very High	Very High
	PP2A	Moderate	High
Brevetoxins (NSP-toxins)	Mouse bioassay	Low	Low
	Neuroblastoma assay	Moderate	Moderate
	Receptor binding assay	Moderate	Moderate
	HPLC-FD	High	High
	LC-MS	Very High	Very High
Yessotoxins	Mouse bioassays	Low	High
	ELISA	High	High
	HPLC-FD	High	High
	LC-MS	Very High	Very High
Pectenotoxins	Mouse bioassay	Low	Low
	HPLC-FD	High	High
	LC-MS	Very High	Very High
	LC-MS	Very High	Very High
Azaspiracids	Mouse bioassays	Low	Low
	LC-MS	Very High	Very High

In the evaluation of testing methods a distinction should be drawn between effect and instrument based methods (Truman *et al.* 2000). Effect based assays measure the response of a biological system (e.g. mouse bioassays, tissue culture assays, enzyme inhibition assays) to the extract whereas instrumental methods (e.g. LC-MS) measure precise quantities of specific toxin molecules.

An advantage of effect based assays is that they measure overall toxicity without the need for knowledge of exactly what toxin variants are present in a sample, as long as they share the same or a similar mechanism of toxicity. The shellfish extraction procedure determines which specific toxins are applied to the assay system, however attempts to develop a universal extraction procedure which will screen for the effect of all toxin groups (e.g. PSP and NSP toxins) using a single assay (e.g. mouse bioassay) have been unsuccessful. Effect based assays may be calibrated with a toxin standard of known concentration (e.g. the PSP mouse bioassays) or the results may be expressed as mouse units (MU) (i.e. the amount of toxin required to kill a standard mouse in a defined time) as in the NSP ether extraction mouse bioassay.

Instrumental analyses (e.g. LC-MS) are more specific than biological methods and lend themselves to precise quantification of specific toxins. A good knowledge of toxin chemistry is required, as the analyst needs to know what variants to expect. Rigorous method performance guidelines including accuracy, specificity, precision, sensitivity and reliability criteria for the analysis of the various toxin groups in a variety of shellfish species and products have to be followed. Central to the success of instrumental analysis is the availability of highly purified and precisely quantified analytical standards. Reliable supplies of certified analytical standards for a number of toxin groups (e.g. yessotoxins and pectenotoxins) are not yet commercially available. Modern LC-MS instruments are capable of simultaneously screening for a wide variety of toxin compounds with a high rate of automated sample throughput. The very high sensitivity of modern LC-MS analysis will undoubtedly reveal that contamination of shellfish with low levels of marine biotoxins is more common than formerly realised and re-evaluation of appropriate regulatory action levels may be necessary in the future.

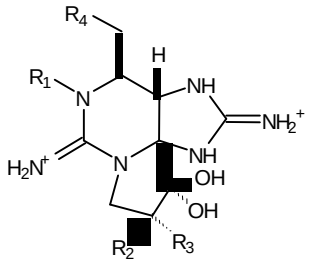
Immunological assays, usually in an enzyme linked, competitive immunosorbent (ELISA) format have characteristics of both effect based and instrument based tests and may in the future become the preferred initial screening method for some toxins (e.g. PSP-toxins and domoic acid). The antibodies at the heart of the assays may be highly specific for a particular toxin molecule or may cross react with a number of closely related toxin variants. ELISA assays are generally highly sensitive and have considerable advantages in that they are relatively cheap and highly portable, may be quantitative or semi-quantitative (i.e. over or under action level only), are easy to use and do not require expensive laboratory facilities. ELISA for domoic acid and PSP toxins (Mist Alert™) are close to completing USFDA validation trials, but have not yet been certified as official regulatory methods. The "DSP-Check" ELISA kit (SCETI Corp) for okadaic acid and other DSP-toxins has been used for some years in New Zealand (in the absence of any other satisfactory method) for the confirmation of DSP-toxin contamination.

4.2 Water Soluble Toxins

4.2.1 Paralytic Shellfish Poisons

Toxin chemistry

The first PSP toxin to be chemically characterised was named saxitoxin after the butterclam *Saxidomus* from which it was isolated. Since then, at least 20 other toxins have been identified from microalgae and shellfish. These toxins all resemble the parent molecule saxitoxin but differ in the type and localisation of derivation (Figure. 1) PSP toxins can be grouped conveniently into carbamate toxins (STX, neoSTX, GTX1, GTX2, GTX3, GTX4), N-sulpho carbamate toxins (GTX5, GTX6, C1, C2, C3, C4) and decarbamoyl-gonyautoxins (dc-GTX). Tasmanian shellfish contaminated by *Gymnodinium catenatum* contain predominantly toxins C1, C2, C3 and C4 (Oshima *et al.* 1987). Port Phillip Bay mussels contaminated by *Alexandrium catenella* also contain predominantly C1-C4 toxins (Arnott 1998), while Adelaide mussels contaminated by *A. minutum* contain GTX1, GTX2, GTX3 and GTX4 (Oshima *et al.* 1989). New Zealand *A. minutum* populations are unusual in that, in addition to GTX1, GTX2, GTX3 and GTX4, they produce significant quantities of neoSTX and STX (Mackenzie & Berkett 1997). Low PSP concentrations have also been found in the gut of Victorian abalone and rock lobsters (Arnott 1998). These different PSP toxins show widely different toxic potencies when injected intraperitoneally into mice, ranging from 2045 MU / μmole (STX) to 16 MU / μmole (C1), in which 1 MU is the amount of toxin to kill a mouse weighing 20 g in 15 minutes upon intraperitoneal injection.



		R4:	—O—C(=O)—NH2	—O—C(=O)—NHSO3−	—OH
R1	R2	R3			
H	H	H	STX	GTX5 (B1)	dcSTX
H	H	OSO_3^-	GTX2	C1	dcGTX2
H	OSO_3^-	H	GTX3	C2	dcGTX3
OH	H	H	NEO	GTX6 (B2)	dcNEO
OH	H	OSO_3^-	GTX1	C3	dcGTX1
OH	OSO_3^-	H	GTX4	C4	dcGTX4

Figure 1. PSP toxins. The parent molecule (left) and derivations (right).

Toxicology and action level

The ultimate toxicity of shellfish to humans depends on the abundance and toxic potency of the microalgae being filtered, and on the chemical transformations of the various toxins, either by the shellfish or during food storage, processing and digestion by human consumers. In humans, 120 to 180 µg PSP STX-equiv. can produce moderate symptoms; 400 to 1060 µg PSP STX-equiv. may cause death, but 2,000 to 10,000 µg PSP STX-equiv. (2 to 10 mg) is more likely to constitute a fatal dose, with the body weight of the patient being an important variable. While the predominance of N-sulpho carbamate toxins in Tasmanian and Port Phillip Bay shellfish suggests a low health risk to humans, these N-sulpho carbamate toxins can easily be transformed under mildly acidic conditions to the corresponding carbamate toxins. C1 thus transforms into GTX2, C2 into GTX3, C3 into GTX1, and C4 into GTX4, with a concomitant 10 to 100 fold increase in toxicity. For this reason, the C1-4 toxins are often referred to as cryptic PSP toxins. While these conversions are easily accomplished *in vitro* in the laboratory, it is not known under what conditions these conversions may also occur *in vivo* in the human stomach. Therefore, until the precise fate of these toxins can be determined, the most conservative toxin regulatory level (80 µg saxitoxin equivalent per 100 g shellfish meat) has been internationally adopted. This is based on an observed lethal level in human adults of 10,000 µg STX equiv., with moderate symptoms appearing at 1,000 µg STX-equiv. (which can be the result of eating, for example, 12 clams weighing 100 grams at toxin levels of 80 µg STX-equiv. per 100 g of tissue). This USFDA quarantine level (quoted as 0.8 mg/kg) has also been adopted by ANZFA as the regulatory limit in the ANZFA Food Standards Code. When seafood products reach this level, the affected area should be closed to both recreational and commercial shellfish harvesting and not reopened until levels decline. The Asia Pacific Economic Co-operation (APEC) has established the principle of performance based criteria for regulatory purposes. That is, the ability to reliably determine whether the total PSP toxins present are below or above the regulatory level of 80 µg STX-equiv./100 g is the ultimate criterion for choice of a particular analytical method.

Mouse Bioassay

To date, the AOAC mouse bioassay (AOAC 1990) is the only internationally accepted method for PSP toxins. In this bioassay, 100 g of shellfish meat is macerated in a blender, gently boiled for 5 minutes with 100 ml 0.1 N HCl, and 1 ml of the clarified extract (pH adjusted to 2.0-4.0) injected intraperitoneally into a 20 g test mouse. The toxicity of the extract is established by measuring the time from injection to the mouse's last breath, using a table of dose/death time relationships and correcting for the precise weight of the test animal. Estimating doses can result in long or short death times leading to substantial errors; extracts therefore need to be diluted by trial and error to achieve death times in the range of 5-7 minutes. Test results are expressed as mouse units (MU) or

calibrated against pure saxitoxin and expressed as micrograms of saxitoxin equivalents per 100 g of shellfish meat (µg STX-equiv./100 g). The method is relatively easy to perform and requires no special equipment assuming a source of suitable laboratory mice is available. The major disadvantage is its poor precision (± 20%) and insensitivity (detection limit is 50 µg STX /100 g).

HPLC analysis

The most successful chemical analysis methods involve the alkaline oxidation of PSP toxins to fluorescent derivatives using periodic acid in sodium phosphate buffer, separation by high performance liquid chromatography (HPLC) and detection by fluorimetry. The HPLC methods developed by Sullivan *et al.* (1983) and Oshima *et al.* (1989) have had a widespread following. The first method uses a polymer PRP column and gradient elution to separate the 10 most common PSP toxins in a single 20 minute run. The second method uses a C8 bonded silica gel column and isocratic elution to separate all known 20 or so PSP toxins in three separate chromatographic runs for N-sulpho carbamate toxins, gonyautoxins and saxitoxins respectively. Shellfish with simple toxin profiles (e.g. from *A. minutum*) can be adequately analysed with the Sullivan method, whereas complex toxin profiles (e.g. from *G. catenatum*) can be resolved only with the Oshima method. HPLC methods offer increased sensitivity (10-20 µg/100 g) and increased precision (5-10%) compared to mouse bioassays, and can operate continuously with automated injection systems. These chemical methods still require extensive calibration against mouse bioassays before they can become accepted as regulatory methods. At the Tasmanian Department of Health, the HPLC method is used for routine monitoring purposes, but positive results still need to be confirmed by mouse bioassay before harvesting of shellfish can be prohibited.

Neuroblastoma assays

The tissue culture assay based on the blocking of sodium channels in mouse neuroblastoma cells has promise as a screening method for PSP toxins. Research in New Zealand (Truman 2000) has shown the test has a detection level of <10 µg/100 g which is sufficiently sensitive to meet regulatory requirements. t has a reproducibility of 15-20%, appears to be specific for PSP-toxins only, and has a good rate of sample throughput with adequate turnaround times achievable. Generally false negative and false positive results are not a problem though recently it has been found that there is a poor correlation between the neuroblastoma assay and mouse bioassays carried out on shellfish contaminated with *Gymnodinium catenatum* toxins in New Zealand (Penny Truman, ESR, New Zealand, pers. comm.). Therefore the assay clearly needs more research and validation before it can be considered as an acceptable testing option.

A mouse neuroblastoma cell bioassay kit (MIST™ kit) for PSP (limit of sensitivity is 2 µg/100 g) was developed by Jellett Biotek Ltd (Jellett *et al.* 1992). However, the limited shelf life of such cell-based assays (1-3 weeks) and false positive results due to interfering substances have been found to cause problems and this kit is no longer in production.

Immuno-assays

An ELISA test kit for PSP is marketed as RIDASCREEN® by R-Biopharm, Germany, while a SAXITOXIN TEST® kit that was produced by Institut Armand-Frappier, Quebec, Canada is no longer in commercial production.

Jellett Biotek (Canada) has recently developed a rapid semi quantitative immuno test kit (MIST Alert™) for the detection of PSP-toxins. This is currently undergoing validation trials.

Receptor binding assays
Radioreceptor binding assays for PSP (Doucette *et al.* 1991) and a method based on the saxitoxin binding protein saxiphilin (Negri and Llewellyn 1998) are also being developed.

LC-MS analysis
Because of the highly polar nature of the PSP-toxins, they present special analytical difficulties for chemists using the ion-spray mass spectrographic technologies that have recently become available and are so successful for the analysis of the low polarity lipid-soluble toxin groups. So far there is no suitable technique in routine use, but work on coupling capillary electrophoresis chromatography and hydrophilic interaction LC with ion spray MS detection are promising techniques (Quilliam 2000). In the future very sensitive and accurate MS analysis of PSP-toxins in shellfish will be possible as a routine screening method.

4.2.2 Amnesic Shellfish Poisons

Toxin chemistry
The causative compound domoic acid (DA) is an excitatory amino acid (Figure 2) acting as a glutamate antagonist on the kainate receptors of the central nervous system

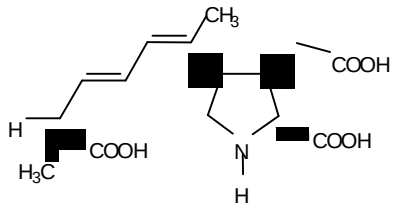


Figure 2. Domoic acid.

Action level.
For the moment the proposed regulatory level in Canada of 20 μg/g tissue (AOAC 1991) has been adopted by other countries screening for the toxin, and has also since been incorporated into the ANZFA Food Standards Code (expressed as 20 mg DA/kg). This is based on the observation of an effect on certain consumers at an estimated domoic acid concentration of 200 μg/g wet weight, with a factor of 0.1 applied for safety reasons (Health and Welfare, Canada). It is recognised that isomers of domoic acid also exist and may contribute to toxicity.

Detection of ASP.
APEC has established the principle of performance-based criteria for regulatory purposes. That is, the ability to reliably determine whether domoic acid is present below or above the regulatory level of 20μg/g is the ultimate criterion for choice of a particular analytical method. There are several methods currently available that meet this criterion.

Mouse bioassays
When ASP was first discovered in Canada, domoic acid was extracted from shellfish using the standardised extraction procedure for mouse bioassay of PSP toxins (Lawrence *et al.* 1989), but with longer observation times (up to 4 hrs). At domoic acid levels >40 μg/g, mice exhibit characteristic scratching symptoms, but this bioassay method is now generally considered not sensitive enough to accurately estimate the proposed action level of 20 μg/g tissue (AOAC 1991).

HPLC analysis

HPLC is now the preferred analytical technique for the determination of domoic acid in shellfish (Lawrence *et al.* 1989, Pocklington *et al.* 1990). Domoic acid is extracted from shellfish tissues by homogenization with methanol-water (1:1, v/v). The concentration of domoic acid is determined by HPLC with ultraviolet absorbance detection. Sample extracts are injected following dilution and filtration of the crude extract or after cleanup on strong anion exchange (SAX) solid phase extraction (SPE) cartridges. The latter provides selective isolation of domoic acid and related compounds from interfering substances such as tryptophan, as well as pre-concentration to facilitate analysis of trace levels. A photodiode array detector can be used to examine UV spectra in order to confirm domoic acid, but this option may not always be available. The detection limit using this method is 20-30 ng/g (ppb). A very sensitive procedure, based on reaction with 9-fluorenylmethylchloroformate (FMOC) to form the fluorescent derivative has been developed for monitoring of domoic acid in seawater and phytoplankton (Pocklington *et al.* 1990). The detection limit is as low as 15 pg/ml and this procedure has recently been adapted to shellfish tissue extracts (Quilliam *et al.* 1995).

LC-MS

Domoic acid can also be tested for by LC-MS (Quilliam et al. 1995). This may offer a cost saving in testing procedures in that it could be included in the extraction used for a DSP screen (Paul McNabb, Cawthron Institute, New Zealand, pers. comm.).

ELISA assay

Jellett Biotek (Canada) have recently developed a rapid semi-quantitative immuno test kit (MIST Alert™) for the detection of domoic acid. This is promising as a means of rapidly screening shellfish for domoic acid contamination and is currently undergoing validation trials.

4.3 Lipid Soluble Toxins

Many of the marine biotoxins are lipid-soluble compounds. Most of these compounds listed in Table 9 are discussed here.

4.3.1 Okadaic Acid and Dinophysistoxins

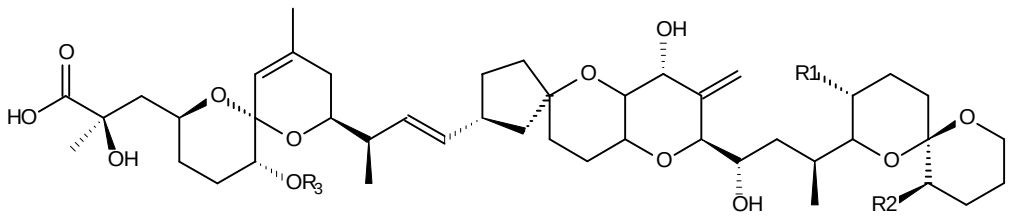
Toxin chemistry

These toxins (Figure. 3) are polyether compounds, based on the parent molecule okadaic acid (OA), which are responsible for the diarrhetic shellfish poisoning (DSP) syndrome. Dinophysistoxins DTX1 and DTX2 are specific compounds with different methylation patterns whereas "DTX3" is not a single compound but a group of chemically related 7-O-acyl esters of OA, DTX1 and DTX2, apparently resulting from enzymatic conversion of these compounds within shellfish tissues (Vale and Sampayo 1999). 'DTX3s' are relatively unstable (they can be easily converted to OA and/or DTX1 and 2 by alkaline hydrolysis), have significantly lower toxicity that OA and DTX1 and have been observed as a minor component of the DSP-toxin complex in New Zealand shellfish (Mackenzie *et al.* 2000a). DTX4 and DTX5 are believed to be precursor molecules in dinoflagellate cells (Wright and Cembella 1998) and in themselves probably do not contribute significantly to the DSP-toxin contamination phenomenon.

Table 9. Lipophilic marine biotoxins found in shellfish

DSP toxins	**NSP toxins**
OA	PbTx-1 (BTX-A)
DTX1	PbTx-2 (BTX-B)
DTX2	PbTx-3 (BTX-B)
DTX3 (7-O-acyl esters of OA and DTXs)	PbTx-10 (BTX-A)
DTX4	BTX-B1
DTX5	BTX-B2
	BTX-B3
OA diol esters	BTX-B4
	Hemi-BTX's A, B & C
Pectenotoxins	
PTX1	**Miscellaneous toxins**
PTX2	Azaspiracid (s)
PTX2sa	
7 epi PTX2sa	**Bio-active compounds**
PTX3	Gymnodimine(s)
PTX4	Spirolide(s)
PTX6	"Wellington Harbour" toxin
PTX7	
	Others (not discussed here)
Yessotoxins	Coolia-toxin
YTX	Goniodomin
45-OH-YTX	Ostreocin
1-desulfoYTX	Pinnatoxin(s)
45,46,47-trinor-YTX	Prorocentrolide(s)
homo-YTX	
45-hydroxyhomo-YTX	
Adriatoxin	

In New Zealand, most valid positive tests for okadaic acid and dinophysistoxin contamination of shellfish (i.e. positive DSP/NSP screen mouse bioassays confirmed by the DSP-Check ELISA), have come from a few locations where the contamination can be positively attributed to *Dinophysis* spp. blooms (Mackenzie *et al.* 2000). Some, primarily 'benthic', members of the dinoflagellate genus *Prorocentrum* also produce DSP-toxins (Rhodes *et al.* 1995, Suzuki and Rhodes 1999) and have been responsible for okadaic acid contamination in oysters in estuarine environments in Northland, New Zealand (Rhodes *et al.* 2001).



R1 = CH3, R2 = R3 = H	Okadaic acid (OA)
R1 = R2 = CH3, R3 = H	DTX1
R1 = H, R2 = CH3, R3 = H	DTX2
R1, R2 = H or CH3, R3 = acyl	DTX3

Figure 3. Okadaic acid and dinophysistoxin parent molecule and derivations.

In Queen Charlotte Sound, New Zealand *Dinophysis acuta* has been shown by HPLC-FD and LC-MS analysis (Mackenzie *et al.* 1999), to produce okadaic acid, but no detectable traces of any of the DTX group. LC-MS screening for 9 individual lipid soluble toxins in cell concentrates of *Dinophysis acuminata* from Port Underwood on the other hand showed no okadaic acid but low levels of DTX1 (Mackenzie *et al.* 2000). Different bivalves respond to exposure to DSP-toxins in different ways. For example Greenshell™ mussels appear to have a unique ability to transform the okadaic acid derived group of DSP toxins to, as yet, unidentified derivatives (Mackenzie *et al.* 1997, Mackenzie *et al.* 1999).

Mouse bioassay

The standard method for the detection of DSP-toxins, based on the 'Yasumoto' (Yasumoto *et al.* 1978) mouse bioassay, has been widely used for nearly 20 years. In New Zealand, Hannah *et al.* (1995) modified the extraction procedure, to include a dichloromethane partitioning step. This produces an extract of superior quality, which is quicker and more sensitive and is used as a combined DSP/NSP screen test that is suitable for the detection of several toxin groups. In New Zealand, if mice deaths occur (2 out of 3 mice dead in 24 hrs), confirmation that the observed toxicity is due to the OA/DTX toxins, has been routinely carried out by using the "DSP-Check" ELISA kit. Recently (February 2001) the use of LC-MS analysis as confirmation has been provisionally approved (Phil Busby, MAF, New Zealand, pers. comm.). Unfortunately the mouse bioassay occasionally returns positive results for which there are no obvious culprits in the plankton and no known toxins can be detected using a range of analytical methods (see section 2.6).

Action level

The regulatory level in shellfish in the ANZFA Food Standards Code for okadaic acid or any dinophysistoxins in shellfish is not equal to or greater than 20 µg/100g in edible part of shellfish.
(Author's note: In November 2001 the EC released new guidelines for the DSP toxins. The regulatory limit for total content of Okadaic acid, Dinophysistoxins and Pectenotoxins is fixed at 16 ug/100 g).

The ELISA assay

The "DSP-Check" assay for DSP toxins (marketed by SCETI Co Ltd Japan) is a competitive enzyme-linked immunosorbent assay (ELISA) which uses monoclonal antibodies with a high specificity against okadaic acid. The manufacturers do not specify how reactive it is against other DTXs though it is thought to have some activity against DTX1. It does not react against DTX3. The manufacturers claim the test has a detection range of 10 ppb-300 ppb (1-30 µg/100 g). Dilutions are required at higher concentrations of okadaic acid. The "DSP-Check" assay has been successfully used in New Zealand for a number of years to confirm okadaic acid contamination of mussels, though it has failed to detect shellfish contaminated with DTX3. An alkaline hydrolysis step in the extraction procedure can overcome this problem (Ian Garthwaite, Ag-Research, New Zealand, pers. comm.) and new test kits using new antibodies in multi-well format are currently being developed by Ag-Research NZ.

HPLC-FD methods

The HPLC-FD (high performance liquid chromatography with fluorescence detection) methods of Lee *et al.* (1987) and Akasaka *et al.* (1996) are both sensitive analyses employing different fluorescent labelling agents which are capable of accurately measuring concentrations of the various DSP-toxins in shellfish. Both methods are time consuming because they involve relatively elaborate clean-up and derivatisation procedures and for this reason they are not ideal as routine marine biotoxin screening methods.

PP2A method

The fluorescence protein phosphatase (PP2A) enzyme inhibition assay (Mountfort *et al.* 1999 and 2001) detects OA and DTX1 down to a level of 1 µg/100 g of mussel tissue making it at least as sensitive as the HPLC-FD analysis and ELISA assay. The PP2A assay does not react to ester derivatives of okadaic acid and DTX1 (i.e. DTX3) but this problem has been overcome by including an alkaline hydrolysis step in the extraction procedure which converts DTX3 to either OA or DTX1 prior to assay (Rhodes *et al.* 2001). This procedure is rapid and cheap, but it has yet to be validated and accredited as a regulatory method.

LC-MS analysis

Liquid chromatography coupled with mass spectrometry has considerable potential as the method of choice for the routine analysis of toxins in the DSP group, if the high capital cost of the instrumentation can be justified. The great advantage of this approach is that multiple toxins can be rapidly and simultaneously identified and accurately quantified with the minimum of sample preparation. There is a major focus on the development of methods for LC-electrospray ionisation MS analysis for DSP toxins by a number of research groups and there are several recent publications describing these. They include Suzuki & Yasumoto (2000) who describe a method for the detection of OA, DTX1 and PTX6 in bivalves; Goto *et al.* (2001) who demonstrate the simultaneous quantification of ten toxins variants; and Quilliam *et al.* (2000) who describe the simultaneous analysis of a wide range of toxins (13) in a blend of contaminated mussel tissue extracts. It is certain that very soon there will be robust methods for the analysis of all known phycotoxins using LC-MS. According to Quilliam *et al.* (2000) "The remaining limitations of a multi-toxin approach lie not in the LC-MS system but in sample preparation- i.e. finding a universal extraction solvent and cleanup scheme that gives good recovery of all toxins." Within the DSP group certified standards and reference materials are available for okadaic acid and DTX1, not for DTX2 or the 'DTX3' variants however.

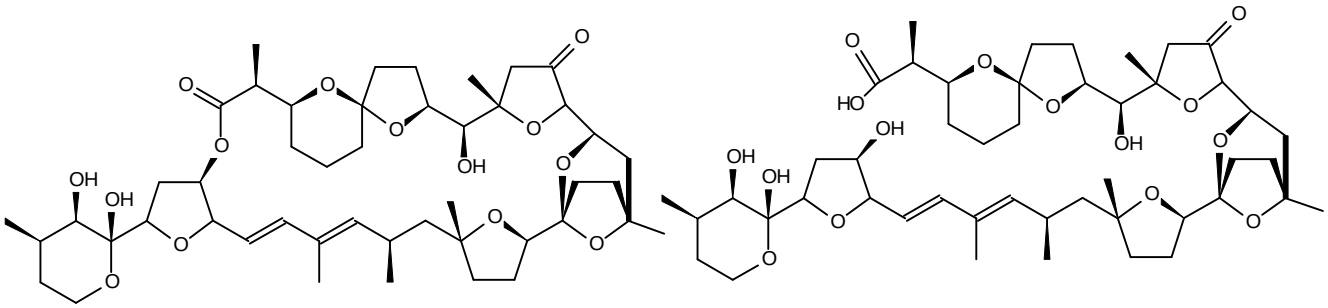
4.3.2 Pectenotoxins

Within the pectenotoxin group, the PTX2 molecule (Figure 4) appears to be the parent compound in Australia, New Zealand and other countries. It is the only pectenotoxin found in the causative dinoflagellates (*Dinophysis* spp.). There is good evidence (Suzuki *et al.* 1998, Suzuki *et al.* 2001) that all other analogues (PTX1, PTX3, PTX6, PTX2sa etc.) are produced as metabolites within bivalve tissues. Different shellfish species clearly transform PTX2 differently. For example, PTX6 has only ever been found in the Japanese scallop (*Patinopecten yessoensis*), whereas in the New Zealand scallop (*Pecten novaezelandae*) PTX2 is converted to PTX2sa, and 7-epi-PTX2sa (Suzuki *et al.* 2001).

Pectenotoxins (specifically PTX2sa) are the possible cause of several instances of shellfish poisoning that occurred following the consumption of pipis from New South Wales beaches between December 1997 and March 1998 (Graeme Arnott, QualSafe Seafood Services, Victoria, Australia, pers. comm.). These incidents were associated with blooms of *Dinophysis acuta* and *D. caudata.* DSP/NSP mouse bioassays screen tests on pipi extracts were positive but DSP-Check ELISA assays were negative. The presence of PTX2sa was confirmed by LC-MS analysis (Quilliam *et al.* 2000) however it has yet to be definitively established that PTX2sa was the cause of the poisoning and other possibilities have not yet been eliminated (e g. the possible role of DTX3).

In New Zealand, both *D. acuta* and *D. acuminata* have been shown to produce significant quantities of PTX2, but none of the other pectenotoxin analogues (Mackenzie *et al.* 1999; Mackenzie *et al.* 2000). The pectenotoxin derivatives PTX2sa and 7-epi-PTX2sa were first simultaneously isolated from New Zealand mussels and *D. acuta* cell concentrates from Ireland (Daiguji *et al.* 1998). Subsequently Suzuki *et al.* (2001) showed that the PTX2 originating in the dinoflagellate is rapidly

converted within New Zealand Greenshell™ and Blue mussels to PTX2sa and 7-epi-PTX2sa, though the latter is always a minor component. Although rigorous toxicological work on the pectenotoxin group has yet to be completed it is known that the cyto-toxicity (i.e. against tissue culture cells) of PTX2sa and 7-epi-PTX2sa is very much less than PTX2 (Daiguji *et al.* 1998). The specific toxicity by oral administration of any of the pectenotoxin group is unknown.



Pectenotoxin-2 (PTX2) Pectenotoxin-2 seco acid (PTX2sa)

Figure 4. Pectentotoxin-2 (left) and pectenotoxin- 2 seco acid (right).

Action level

Pectenotoxins are regulated for in the DSP test and the regulatory level in shellfish in the ANZFA Food Standards Code is not equal or greater than 20 µg/100 g in edible part of shellfish.

(Author's note: In November 2001 the EC released new guidelines for the DSP toxins. The regulatory limit for total content of Okadaic acid, Dinophysistoxins and Pectenotoxins is fixed at 16 ug/100 g.)

HPLC-UV analysis

The HPLC-UV/DAD method of Draisci *et al.* (1996) has been used to measure pectenotoxin concentrations in shellfish tissues however it lacks sensitivity and needs further development to be suitable for use as a routine analytical method.

LC-MS analysis

There are several recent publications (Suzuki *et al.* 1998, Draisci *et al.* 1999, James *et al.* 1999, Suzuki & Yasumoto 2000 and Suzuki *et al.* 2001), which illustrate that HPLC-electrospray ionisation MS is unquestionably the method of choice for the rapid, sensitive and definitive analysis of pectenotoxins, though as yet no method has been officially validated for regulatory use. The current unavailability of analytical standards and reference materials for any of the pectenotoxins is an impediment to the adoption of LC-MS as a regulatory method though hopefully this will be solved in time. The Cawthron Institute has small quantities of pure quantified PTX2 and good quantities of PTX2, PTX2sa and 7-epi-PTX2sa contaminated shellfish reference materials which it has made available to Australian analysts in the past. It is expected that further quantities will available in the future.

4.3.3 Yessotoxins

The yessotoxin (YTX) molecule resembles the brevetoxins and ciguatoxins in that it has a ladder frame polycyclic ether skeleton. It does not have the neurological activity of these toxins however, and although it is lethal and causes heart and liver pathologies when administered to mice by intraperitoneal injection its oral toxicity remains questionable (Terao *et al.* 1990). Yessotoxin was first described in extracts of the Japanese scallop *Patinopecten yessoensis* in the 1980s (Murata *et*

al. 1987) but its origin has only recently been identified as the planktonic dinoflagellate *Protoceratium reticulatum* from research carried out in New Zealand (Satake *et al.* 1997). To date at least eight analogues of the parent yessotoxin molecule have been isolated and described from a variety of shellfish species around the world. These are 45-OH-YTX, 1-desulfoYTX, 45-, 46-, 47-trinor-YTX, homo-YTX, 45-hydroxyhomo-YTX and adriatoxin. Several yessotoxin contamination incidents have been documented in New Zealand, one of which resulted in the destruction of a substantial amount of harvested shellfish products (McCoubrey 1998). There are no known incidents involving yessotoxin contamination in Australian waters, all though it almost certainly occurs from time to time as the causative dinoflagellate is a common inhabitant of temperate coastal waters.

Action level
Yessotoxins are regulated for in the DSP test and the regulatory level in shellfish in the ANZFA Food Standards Code is not equal to or greater than 20 µg/100 g in edible part of shellfish. This level is currently under review by the EC DSP expert group.
(Author's note: In November 2001 the EC released new guidelines for the DSP toxins. The regulatory limit for Yessotoxins is fixed at 100 µg of yessotoxin equivalent/100 g.)

Mouse bioassay
The symptoms of bioassay mice inoculated with yessotoxin contaminated material include rapid death times, gasping, general weakness and fitting prior to death.

ELISA assay
An ELISA assay is currently under development by the toxinology research group Ag-Research (NZ) but requires extensive validation trials before it can be considered for adoption as a routine analytical method.

HPLC-FD analysis
The HPLC-FD method of Yasumoto & Takizawa (1997) incorporating the modifications of Suzuki & Mackenzie (1999) for the measurement of yessotoxins in shellfish has proven very useful for research purposes but it is relatively slow and in its present form is not suitable for use as a routine analytical method.

LC-MS analysis
The LC-MS method based on that of Draisci *et al.* (1998) is a highly sensitive and definitive method of screening for yessotoxin and its derivatives (e.g. 45-OH-YTX) in shellfish samples, although as yet neither it, nor any other method has been validated and officially certified. The current unavailability of analytical standards and reference materials for any of the yessotoxins is an impediment to the adoption of LC-MS as a regulatory method. The Cawthron Institute is currently producing sufficient quantities of yessotoxin for the production of a standard. Problems regarding the instability of purified yessotoxin need to be overcome before a certified standard can be produced, however it is anticipated that an interim (i.e. quantified though uncertified) standard will be available by 2002.

4.3.4 NSP Toxins

Until relatively recently, neurotoxic shellfish poisoning (NSP), caused by polyether brevetoxins (BTXs), also known as '*Ptychodiscus brevis*-toxins' (PbTxs) from the unarmoured dinoflagellate *Karenia brevis (=Gymnodinium breve, =Ptychodiscus brevis*), was considered to be endemic to the Gulf of Mexico and the East Coast of Florida. Unexpectedly, in early 1993 more than 180 human shellfish poisonings were reported from New Zealand, caused by an organism similar (but not identical) to *Karenia brevis (=Gymnodinium breve)* (Jasperse 1993, Haywood *et al.* 1996). Similar

dinoflagellates have also been identified in low concentrations from Victorian, South Australian and West Australian waters. Recent evidence suggests that blooms of the raphidophyte *Chattonella marina* (e.g. in Port Lincoln in April 1996; Hallegraeff *et al.* 1997), and possibly the related genera *Fibrocapsa* and *Heterosigma*, can also produce brevetoxin-like compounds (Kahn *et al.* 1996a, 1996 b, 1997).

BTX's and their derivatives exert their toxic effect by specific binding to, and activation of, sodium channels in nerve membranes. In humans, the symptoms of NSP intoxication include neurological symptoms, respiratory distress, eye and nasal membrane irritation, and are caused principally by exposure to sea-spray aerosols and by direct contact with toxic algal blooms while swimming. No human fatalities from brevetoxin poisoning have ever been reported.

Toxin chemistry

Brevetoxins are a group of lipid-soluble, ladder-form polyether toxins. Many of these compounds have been characterised, but assay and analysis continues to be difficult. Brevetoxins based on the BTX-A molecular skeleton as produced by the Florida *K. brevis* has not been found in New Zealand shellfish. Ishida *et al.* (1996) isolated PbTx-2 and PbTx-3 (both or which are based on the BTX-B molecule) from New Zealand Pacific oysters (*Crassostrea gigas*) in January 1993. PbTx-3 was also isolated from New Zealand Pacific oysters in February 1994 and June 1995. Ishida *et al.* (1995) isolated and identified a novel toxin (BTX-B1) from New Zealand cockles (*Austrovenus stuchburyi*) in January 1993. Murata *et al.* (1998) and Morohashi *et al.* (1995) isolated and identified BTX-B2 and BTX-B3 from New Zealand Greenshell^{TM} mussels in January 1993. BTX-B3 did not kill mice by intraperitoneal injection and it is likely that the mussels transform BTX-B1 to the less toxic BTX-B3. BTX-B2 retained intraperitoneal toxicity and sodium channel activity (about 1/3 as potent as PbTx-3) but was not ichthyotoxic (i.e. toxic to fish). Morohashi *et al.* 1999 isolated and identified BTX-B4 from New Zealand Greenshell^{TM} mussels in January 1993. This analogue, which is a derivative of BTX-B2, accounted for nearly two-thirds of the mouse lethality of the shellfish. BTX-B, PbTx-3 and BTX-B1 (which was found in cockles during the same period) were not present in these mussels which suggests that bivalves metabolise the brevetoxins in a species specific manner.

Action levels

In Florida and North Carolina, shellfish harvesting is suspended when cell concentrations of *K. brevis* exceed 5,000 cells/L or seafood toxins exceed 20 MU /100 g. New Zealand has also adopted this latter regulatory level, but are currently reviewing the trigger level used based on review of NSP monitoring in New Zealand. Respiratory problems in humans occur at about 10^5-10^6 cells per litre, while fish kills occur at > 10^6 cells per litre. Levels of NSP during the 1993 New Zealand shellfish poisoning outbreak reached 592 MU/100 g (Trusewich *et al.* 1996). In January 1994, mussels from Tamboon Inlet on the Gippsland coast of Victoria contained 27.5 MU/100 g in association with a *K. brevis* type bloom (analyses by Medvet Science Pty Ltd using the Hannah method (Arnott 1998)). The proposed regulatory limit for NSP toxins in shellfish in the ANZFA Food Standards Code is also equal to or greater than 20 MU/100 g in the edible part of the shellfish.

Mouse bioassays

The currently accepted method for the determination of NSP toxins is the American Public Health Association (APHA 1985) procedure based on diethyl-ether extraction of shellfish tissue followed by mouse bioassay. The APHA protocol is widely used in the United States, where the problem of NSP is most acute. After the detection of NSP in New Zealand in 1993, NZ MAF Regulatory Authority improved the sample preparation method by utilising acetone extraction of lipophilic components, followed by partitioning into dichloromethane (Hannah *et al.* 1995). Sample extracts are prepared for mouse injection, and the bioassay results are calculated in mouse units. The

'Hannah procedure' is very effective in extracting unknown lipid-soluble toxins from shellfish, and the method presents certain advantages compared with the APHA protocol. However, the discovery of gymnodimine (section 3.5.1) has led to local health authorities returning to the APHA diethyl-ether extraction as a confirmatory procedure in the event of mouse deaths with characteristic NSP symptoms. Gymnodimine is not extractable by diethyl ether but it causes very rapid mouse deaths when the dichloromethane procedure is used.

Neuroblastoma assay
The tissue culture assay based on the activation of sodium channels in mouse neuroblastoma cells has promise as a screening method for brevetoxins. The assay is non-specific in that it detects activity rather than detecting specific molecular structures however recent trials (Truman 2000) have shown no evidence of false negatives or positives. The assay is sufficiently sensitive (<2 µg/100 g corresponding to approximately 5 MU/100 g) to detect toxin well below the regulatory level, the reproducibility is ±20% (expected for this type of assay) and running the assay is rapid with a high volume of sample throughput achievable. Because of the need to maintain tissue cultures under highly aseptic conditions this test will probably remain laboratory based though attempts have been made to format it for use in field test kits. Despite its potential the neuroblastoma assay has yet to be used as a regulatory method and requires further performance and validation trials to achieve this.

Radioreceptor binding assays
A sensitive radioreceptor assay for brevetoxins is based on binding to site 5 on the voltage dependent sodium channel in rat brain synaptosomes, using ^{3}H- $PbTx_3$ for quantification (Trainer *et al.* 1995). The assay is a useful research tool but is unlikely to become a routine analytical method

ELISA assays
Promising ELISA assays for NSP-toxins are under development (Garthwaite *et al.* 1996), however, the wide range of potential BTX analogues in contaminated shellfish creates specificity problems for these assays.

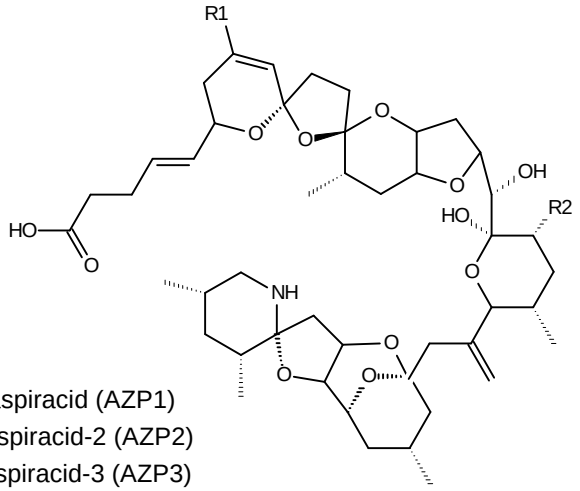
LC-MS analysis
A high performance liquid chromatography-electrospray ionisation mass spectrometry method for the determination of BTX's has been developed (Hua *et al.* 1995) and a method similar to this is likely to become the method of choice in the future. Further basic research and method development is still required however.

Standards and reference materials
Some brevetoxin standards are commercially available, although these do not cover the full range of known analogues.

4.3.5 Azaspiracids

Toxin Chemistry
Azaspiracid shellfish poisoning (AZP) is a newly described syndrome (James *et al.* 2000) involving a suite of toxins named azaspiracids (AZAs) (Figure 5) based on a novel molecular structure. AZ-1 (MW842) has a complex structure involving two spiro ring assemblies and carboxylic acid amine groups (Satake *et al.* 1998). These toxins have caused human poisoning with symptoms including nausea, vomiting, severe diarrhoea and stomach cramps (Satake *et al.* 2000) and have been shown to damage the villi in the small intestine and can cause multiple organ damage in laboratory animals (Ito *et al.* 2000). The toxins cannot reliably be detected at a level that would prevent human illness using the conventional mouse bioassays for DSP toxins, as their activity by intraperitoneal injection is very slow. AZ-1 is the parent toxin but methyl and dimethyl analogues (AZ-2 and 3) have also

been found in contaminated shellfish. It is believed that the origin of AZP is a planktonic dinoflagellate (Kevin James, Cork Institute of Technology, Ireland, pers. comm.) but the causative organism has yet to be positively identified. AZP has never been identified in New Zealand or Australia.



R1 = H, R2 = CH_3 Azaspiracid (AZP1)
R1 = R2 = CH_3 Azaspiracid-2 (AZP2)
R1 = R2 = H Azaspiracid-3 (AZP3)

Figure 5. Azaspiracids

Action level
Currently azaspiracids are not a regulated substance in Australia. In New Zealand shellfish they are regulated for as a DSP toxin, and as such the regulatory level is not equal to or greater than 20 µg/100 g in the edible part of the shellfish. The EC DSP expert group has since reviewed this level and the regulatory limit for Azaspiracids is fixed at 16 µg of azaspiracid equivalents/100 g.

LC-MS analysis
There is only one known method for the analysis of AZP toxins, which is the rapid and highly sensitive LC-MS method of Draisci *et al.* 2000. This method has a detection level of 50 pg with a sensitivity that is about 80,000 times greater than the mouse bioassay. In the event of detecting AZ-1 screening for AZ-2 and AZ-3 can be carried out.

Standards and reference materials
There are no commercially available standards or reference materials for AZP toxins. Limited amounts of purified AZP-toxins may be available from Dr Kevin James, Cork Institute of Technology, Ireland and/or Dr Masayuki Satake, Tohoku University, Japan.

4.4 Bio-active Compounds

These are compounds that are known to cause a reaction in the mouse bioassay, but are not toxins, and internationally are not considered a public health hazard (Phil Busby, MAF, New Zealand, pers. comm.).

4.4.1 Gymnodimine

Gymnodimine was first extracted and identified in dredge oysters (*Tiostrea chilensis*) harvested from Foveaux Strait, New Zealand (Seki *et al.* 1995) in which it accumulates and is retained for considerable periods of time (Mackenzie *et al.* 1996). Recently a new analogue, gymnodimine B, has been identified (Miles *et al.* 1999). Both compounds are produced by the dinoflagellate *Karenia selliformis* (=*Gymnodinium selliforme*) sp. nov. (Haywood *et al.* in press). Gymnodimine

contamination has caused the majority of the positive DSP/NSP screen mouse bioassays within the New Zealand marine biotoxin surveillance program since its inception in 1994. Gymnodimine has a cyclic imine structure common with other marine bioactive compounds such as spirolides. Gymnodimine causes rapid deaths in mice with intraperitoneal injection; however, it appears to have a low level of oral toxicity. There have been no human illnesses associated with very high levels of contamination and at present is not regarded as a public health risk and not regulated for in New Zealand.

4.4.2 Spirolides

The spirolides are a recently identified group of marine bio-active compounds first isolated from the digestive glands of mussels in Canada (Hu *et al.* 1995 & 1996). These compounds are characterised by the rapid onset of symptoms in the mouse bioassay and have molecular structural features (a cyclic imine moiety) in common with other fast acting compounds such as gymnodimine. In Canada spirolides are produced by *Alexandrium ostenfeldii* (Cembella *et al.* 2000), New Zealand isolates of the same species do not produce these compounds (Mackenzie unpublished). It is not known whether spirolide contamination occurs in Australian waters. These or similar, but as yet unidentified compounds, are a potential cause of unexplained positive mouse bioassays where the mice exhibit rapid death times.

4.4.3 "Wellington Harbour Toxin"

This is a fast acting compound which was discovered in mussels contaminated by a bloom of *Karenia brevisulcata* (=*Gymnodinium brevisulcatum)* (Chang 1999) in Wellington Harbour, New Zealand in the summer of 1998. The active compound has yet to be isolated and identified.

4.5 Factors Which Confound Mouse Bioassays for Marine Biotoxins

4.5.1 Aqueous Shellfish Extracts

Mouse bioassays for water soluble toxins (e.g. PSP and ASP) have generally been found to be much less prone to false positive and false negative responses than the lipid soluble toxin group but under some specific circumstances problems have arisen. High levels of metals (e.g. zinc) which may occur naturally in some shellfish (e.g. oysters) can cause the death of mice inoculated with aqueous extracts, and extraction of shellfish with high strength acid (e.g. 1.0 N HCl) resulted in numerous false positive bioassays in New Zealand in 1993.

4.5.2 Lipid Shellfish Extracts

Mouse bioassays for lipid soluble toxins are notoriously prone to providing positive results for which there is no ready explanation and have caused problems in Australia, Ireland, New Zealand and elsewhere. On several occasions in New Zealand, the recall of commercial mussel products because of apparently false positive DSP/NSP screen mouse bioassays has resulted in substantial financial losses to growers (Truman 1997). In some of these incidents shellfish have been screened for all known algal toxin groups and subjected to intensive analysis using LC-MS without any definite cause being established. A characteristic of these events has been the sporadic nature of the occurrence with shellfish being reactive one week but not the next and a casual observation made during these incidents is that the shellfish are in good/fat condition. It is known that free fatty acids in shellfish (Takagi *et al.* 1984, Sajiki & Takahashi 1992, Lawrence *et al.* 1994, Suzuki *et al.* 1996), probably originating from phytoplankton, can cause spurious results in the mouse bioassay. It is suspected that this is the cause of some, if not most, of the unexplained lipid positive bioassays in New Zealand.

5 REVIEW OF EXISTING STATE MARINE BIOTOXIN MONITORING PROGRAMS

5.1 Introduction

A thorough evaluation of all current State and Territory 'Marine Biotoxin Management Plans' was undertaken. Information was collected by way of a questionnaire (Appendix 1) which was distributed to State and Territory Program Managers.
Sources of information made available to conduct the review included:

- State or Territory biotoxin management plans, biotoxin contingency plans and/or biotoxin monitoring programs;
- Other relevant biotoxin documents including 'Memoranda of Understanding', 'Inter-Agency Agreements', etc.;
- Relevant State or Territory legislation;
- Responses by State or Territory Program Managers to the detailed questionnaire on 'Existing State Biotoxin Programs'; and
- Follow-up contacts with Program Managers, via telephone and/or e-mail, for clarification and further detail.

The State and Territory 'Biotoxin Management Plans' were initially summarised in common format to highlight key elements and to enable a quick comparison to be made of current practices between States. The report headings are as follows:

- Date of Latest Plan
- Responsible Agency
- Brief History of Biotoxin Surveillance
- Bivalve Shellfish Resources
- Toxic or Potentially Toxic Algal Species
- Designated Shellfish Growing Areas
- Phytoplankton and Biotoxin Monitoring
- Closure and Re-opening Criteria
- Program Administration
- Internal Reviews

The present program reviews essentially provide a snapshot of biotoxin surveillance operations within Australian waters. Considerable variation was apparent in both the size and quality of the individual programs, and in the quality of biotoxin management documentation, and this variation was dependent on the size of the relevant shellfisheries and on past State and Territory experience of toxic marine algal blooms. All Marine Biotoxin Management Plans were evaluated to assess both strengths and weaknesses, the actual weaknesses, not the number being of most importance. Comments are provided for each weakness, whether major or minor, to assist in the process of continuous improvement. Some program changes were already planned at the time of this review. These reviews generally focus on the cultured shellfish industries, however many of the plans may also be applicable to for use in both commercial and recreational wild harvest areas.

5.2 New South Wales Shellfish Quality Assurance Program (NSW SQAP)

(Authors note: Safe Food Production presented a detailed and comprehensive response to the questionnaire, and much of this material has been reproduced here (McFarlane *et al.* 2000)).

Date of Latest Plan
The 'Marine Algal Biotoxin Management/Reaction Plan', which forms part of the NSW Shellfish Quality Assurance Program (NSW SQAP), was created by Ministerial Determination No. MAB2/1999 signed by the Minister of Fisheries in July 1999. The contingency plan (known as the Marine Algal Biotoxin Contingency/Management Plan) was amended in May 2001.

Responsible Agency
Safe Food Production NSW.
Current Program Manager of NSW SQAP: Dr Kerry Jackson.
Further discussion of the roles and responsibility of relevant associated agencies and shellfish industries is provided in section 4.2.8 Program Administration.

Brief History of Biotoxin Surveillance
The NSW SQAP was created by Fisheries Regulation in May 1995 and program operations first commenced in September 1996. The main objective was to assure the quality and safety of farmed oysters and mussels taken from estuarine and marine waters for sale for human consumption. However, in the first instance the Program was established to control the harvest of oysters and mussels from leases under a Class A Aquaculture permit. More recently, in 1999, the management of the NSW SQAP was transferred from NSW Fisheries to Safe Food Production NSW. The NSW SQAP developed the NSW Marine Algal Biotoxin Management/Reaction Plan. The plan has since been updated and is known as the Marine Algal Biotoxin Contingency/Management Plan. The plan has been activated on 6 occasions since implementation.

The Sydney Rock industry is the oldest and largest fishery in NSW whose origins date back to the turn of the 20th century. Sydney rock oysters are cultivated in many estuaries distributed along much of the NSW coastline, whereas Pacific oysters (an introduced species) can only be farmed legally in Port Stephens. The total lease area available for oyster cultivation in NSW is about 4,500 hectares, although current farming practices are such that not all of this area is continuously utilized from the time of natural spatfall to final harvesting.

The NSW SQAP has been implemented in all lease areas where commercial oyster farming is undertaken, and commercial harvesting is prohibited in any area where the NSW SQAP is not operational. Formal quality assurance procedures have not yet been integrated into the NSW SQAP for the experimental scallop industries. In addition, the program does not yet control the commercial harvest of wildstock shellfish.

Reports of toxic algae, toxic or potentially toxic algal blooms and shellfish poisoning incidences in NSW have been documented in two reports to the NSW Premier's Department in fairly recent years:

1. 'The Management of Marine Algal Biotoxin Issues in NSW' prepared by the NSW Marine Algal Biotoxin Committee, November 1995.

2. 'Report of Marine Algal Technical Group', November 1998.

The reported biotoxin related events are:

Sediment samples collected from oyster growing areas in Botany Bay in 1993 contained resting cysts of the potentially toxic dinoflagellate *Alexandrium* sp. (Lincoln-Smith & Smith 1993). Dinoflagellate cysts and remains (*Alexandrium* and *Dinophysis* spp.) have also been reported in the gut contents of oysters from Port Stephens, a major oyster-producing estuary (Richardson 1991).

- *Alexandrium catenella*, a PSP-producer, was detected for the first time in NSW in Sydney Harbour during November 1993. Samples of wildstock oysters collected during the bloom contained more than 3 mg saxitoxin equivalent per kg, which exceeded the 'Food Standards Code' regulatory limit of only 0.8 mg per kg. Low levels of PSP toxin were also detected in Sydney Harbour prawns. Health warnings were issued advising the public not to consume oysters from Sydney Harbour. *A. minutum* was also detected in the Shoalhaven River at this time.

- The toxic diatom *Pseudo-nitzschia multiseries*, a potential domoic acid producer, was detected during phytoplankton monitoring conducted by the Environment Protection Authority (EPA) in the Berowra Creek in 1993 (Hallegraeff 1994) and again in 1995. Berowra Creek has also experienced blooms of the potentially toxic algae *Karlodinium micrum* (=*Gymnodinium galatheanum*) and *Heterosigma akashiwo*; both species may possibly produce brevetoxin (needs confirmation). The sampling site was not far from major oyster growing and prawn trawling areas in the Hawkesbury River.

- A red algal bloom of *Chattonella* cf *globosa* was observed in Sydney Harbour and the Parramatta River from November 1996 to January 1997. It is possible that this species may also produce brevetoxin, although again confirmation of toxin production is required.

- An outbreak of over 50 cases of gastroenteritis was linked to the consumption of pipis commercially harvested from Ballina in northern NSW in December 1997. The illness was thought to be diarrhetic shellfish poisoning (DSP) by NSW Health. The guts of pipis collected at the time were examined and found to contain *Dinophysis acuminata* and *D. caudata.* Contaminated pipi meat was later tested and the pectenotoxins PTX2 and PTX2sa were detected. However full epidemiological case investigations were not conducted and so these cases remain unconfirmed. If these investigations had been performed, these would have been the first confirmed pectenotoxin shellfish poisoning cases in the world, and would have provided important information worldwide to the management and setting of regulatory levels for pectenotoxins. Pipi harvesting was suspended at Ballina for over two months. In March 1998 an outbreak of over 20 cases of a similar illness was reported following the consumption of recreationally harvested pipis from the Anna Bay/Stockton Beach area near Newcastle. The affected area was closed for several months.

- A bloom of the marine 'blue-green' alga *Trichodesmium* sp. occurred in Batemans Bay during Easter 1998. A mouse bioassay conducted on an algal sample revealed a toxic effect (details not provided), but later HPLC analyses could not identify the actual toxin(s). Commercial oyster harvesting was suspended at this time and the public was warned not to eat shellfish from the bloom area.

- A further potentially toxic bloom, consisting mainly of *Pseudo-nitzschia pseudodelicatissima*, occurred in Berowra Creek in October 1998. Oyster growing areas were affected and the local coordinator of the relevant shellfish quality assurance program closed the area for oyster harvesting for a short period. The NSW EPA and Hornsby Council issued a public warning and closed the 'bloom' area for recreational use. HPLC analyses subsequently confirmed that the

alga was non-toxic; all strains of this species have been consistently non-toxic throughout Australia to date.

Safe Food NSW provided two additional more recent events:

- On 8 October 1999 a fishery closure occurred in Wagonga Inlet due to a bloom of *Pseudo-nitzschia spp*., later found by Gustaaf Hallegraeff (University of Tasmania) to consist of *P. pseudodelicatissima* and *P. pungens.* Oyster harvesting resumed on 21 October 2000 after two negative test results for domoic acid.

- In December 1999 the taking of finfish and shellfish by recreational and commercial fishers was prohibited in specific areas of the Myall Lakes. The closure was deemed necessary due to high levels of potentially toxic blue-green algae consisting mainly of *Anabaena* sp. together with low numbers of *Microcystis* sp.

The NSW 'Marine Algal Biotoxin Management/ Reaction Plan' initially developed and implemented by NSW Fisheries in 1999, was activated several times by Safe Food NSW during 1999-2000 and fifteen times to the end of April 2001 in the current financial year. The plan has since been updated and is known as the Marine Algal Biotoxin Contingency /Management Plan. The plan has been activated on 6 occasions since its implementation in May 2001. Action was taken mainly as a precautionary measure due to the presence of potentially toxic algae or suspect algal blooms. However, although based on a small number of toxin analyses, no biotoxins have yet been detected in NSW commercially farmed oysters or mussels. The Plan is essentially a contingency plan that provides a response capability. The Plan currently depends on the notification of potentially toxic algal blooms by the oyster and mussel culture industries and relevant Government agencies. A routine phytoplankton monitoring programme is conducted in Wallis Lakes (commenced November 2000), mouth of Port Stephens (commenced 1999), Nelson Lagoon and Twofold Bay (commenced April 2001).

Safe Food NSW currently provides no dedicated funding to implement biotoxin management as a component of the NSW SQAP, although it is pursuing funding for biotoxin monitoring from the State Government. However, Safe Food NSW does provide in kind and financial support to the NSW SQAP; the overall Program is funded 60% by industry, 30% by Safe Food NSW and 10% from other sources. When a bloom occurs sampling is conducted by shellfish farmers or by NSW SQAP staff and analytical costs are paid from industry levy funds.

Fisheries NSW have temporarily retained administrative responsibility for biotoxin management of the pipi industry.

Bivalve Shellfish Resources

- Sydney rock oyster (*Saccostrea glomerata*)
- Pacific oyster (*Crassostrea gigas*)
- Native flat oyster (*Ostrea angasi*)
- Blue mussel (*Mytilus edulis*)
- Commercial scallop (*Pecten fumatus*) [roe-on]
- Ballots saucer scallop - *Amusium balloti*) [roe-off]
- Doughboy scallop (*Mimachlams asperrima*) [roe-off]
- Sydney cockle (*Anadara trapezius*)
- Sand cockles (*Katelysia spp.*)
- Pipis (*Plebidonax deltoides* plus *Donax* spp.)

- Surf clams (*Dosinia caerulea* plus *Dosinia* spp.)

Most of the above species are widely distributed in the State and, with the exception of pipis, can all be harvested recreationally by the public for human consumption, however strict bag limits apply. Many Aboriginal groups harvest bivalve shellfish for subsistence purposes. Pipis can only be collected for bait and cannot be taken beyond 50m of the high-tide mark. The gathering of invertebrates is prohibited in Intertidal Protected Areas (IPA) and is either prohibited or restricted in designated 'Aquatic Reserves'. NSW Fisheries currently list the whole of Sydney Harbour and fourteen other areas around Sydney as IPAs. Bag limits apply to bivalves taken from open areas to protect shellfish resources: oysters (50), scallops (50) and cockles, mussels and pipis combined (50).

The Sydney rock oyster, Pacific oyster, native oyster, pearl oyster and blue mussel are the only species currently cultured commercially in NSW.

Commercial scallops, native flat oysters, blue mussels, Sydney cockles and pipis, on the other hand, are commercially harvested. The scallops are dredged in southern NSW waters, while the other species are collected by hand gathering. Commercial fishers in the "Estuary General Fishery" (a restricted fishery) may have endorsements to hand gather on their licence. The number of fishes with endorsements to hand gather, in each of seven coastal regions, is as follows:

1.	Upper North Coast	14
2.	Clarence	3
3.	North Coast	12
4.	Central	13
5.	Metropolitan	0
6.	Upper South Coast	9
7.	Lower South Coast	3

Designated Shellfish Growing Areas

Sydney rock oysters are farmed commercially in 41 estuaries along the NSW coast, extending from Tweed Heads in the north to Wonboyn Lake in the south. However, oysters are not harvested directly for human consumption from all of these estuaries. Many leases are used simply for catching spat or for depoting sticks and early growout. The oysters are generally handled and moved many times during the growout period before being transferred to "harvest leases" for finishing off (fattening) prior to sale. Commercial harvesting of Sydney rock oysters for human consumption only occurs from the following 30 estuaries:

Tweed River	Manning River	Clyde River
Brunswick River	Wallis Lake	Tomaga River
Richmond River	Port Stephens (Zones 1-7)	Tuross Lake
Clarence River	Hunter River	Wagonga Inlet
Wooli River	Brisbane Waters	Bermagui River
Belliger & Kalang Rivers	Patonga Creek	Wapengo Lake
Nambucca River	Hawkesbury River	Nelson Lagoon
Macleay River	Georges River	Merimbula Lake
Hastings River	Shoalhaven & Crookhaven Rivers	Pambula River
Camden Haven	Lake Conjola & Burrill Lake	Wonboyn River

Pacific oysters are farmed only in Port Stephens, in NSW SQAP Harvest Zones 1-7, although this oyster is now also present in other estuaries where it is a noxious pest and is treated as such with notices for destruction issued.

Blue mussels are cultured only in Twofold Bay at Eden.

Doughboy scallops, native flat oysters and pearl oysters are currently the subject of research and trial farming. However, any future commercial cultivation of these species is likely to occur in existing aquaculture permit areas. While the pearl oysters would be cultured for the production of pearls, it may be possible to sell the adductor muscle for human consumption.

Phytoplankton and Biotoxin Monitoring

Monitoring is essentially based on a contingency plan, which provides a response capability. Routine phytoplankton monitoring programmes are conducted in Wallis Lakes, mouth of Port Stephens, Nelson Lagoon and Twofold Bay. Several groups who conduct some relevant microalgal monitoring in specific areas also forward the data to the NSW SQAP. These groups include:

- Regional Algal Coordination Committees, which conduct routine microalgal monitoring in fresh water systems and issue alerts on blue-green algae on a regular basis.
- The pipi harvest industry, which conducts routine phytoplankton and appropriate biotoxin monitoring at specific ocean beaches as required under their individual biotoxin management plans.
- The Newcastle Port Authority, which undertakes weekly microalgal monitoring at eight sites inside and one site outside the Port. Newcastle Port is located at the mouth of the Hunter River, which is an oyster harvest area.
- The Hornsby Shire Council, which undertakes some microalgal monitoring near oyster harvesting areas in the Hawkesbury River.

Safe Food NSW has also recently initiated limited monitoring at several high-risk areas where sampling is to be conducted every 4-6 weeks.

Closure and Re-opening Criteria

Closure Criteria

Shellfish growing areas must be closed for harvesting when the biotoxin concentration in shellfish is likely to cause a public health risk based on the following criteria:

- The concentration of paralytic shellfish poison (PSP) (saxitoxin & derivatives) must not be equal to or exceed 0.8 mg/kg of the edible portion of raw shellfish.
- The concentration of domoic acid must not be equal to or exceed 20 mg/kg of the edible portion of raw shellfish.

These criteria are the same as those specified in the current ‘Operations Manual’ of the Australian Shellfish Quality Assurance Program (ASQAP).

No criteria are provided for toxins responsible for diarrhetic shellfish poisoning (DSP) and neurotoxic shellfish poisoning (NSP). As routine phytoplankton monitoring is not conducted there are also no closure criteria based on the cell concentration of a toxic algal species exceeding a prescribed abundance, pending the results of toxin testing of shellfish meat. However, the “State Coordinator” may close shellfish growing areas, as a precautionary measure, when suspect blooms are observed or when unusual environmental conditions occur which require further investigation.

There is also no specific closure criteria based on the reporting of human illness fitting the accepted case definitions for PSP, ASP, DSP or NSP.

NSW Fisheries has the legislative power to prohibit the taking of fish, or a specified class of fish, from any waters or from specified waters; see Fisheries Management Act 1994, Part 2, Division 1, Section 8 'Closure of waters to fishing'. Any such prohibition is called a 'fishing closure'.

Additional legislative powers are provided by the Fisheries Management (Aquaculture) Regulation 1995 under the Fisheries Management Act 1994. Under Division 4, which establishes the New South Wales Shellfish Quality Assurance Program, all Class A aquaculture permit holders must comply with the requirements of the NSW SQAP. The Program Manager has delegated authority to close aquaculture areas for harvest when environmental conditions indicate that it is not safe to harvest product. This function of the Program Manager will be transferred to the Seafood Safety Scheme Regulation under the Food Production (Safety) Act 1998 in mid 2001.

Safe Food NSW also has legislative powers to prohibit activities or impose conditions in relation to seafood as specified in the Food Production (Safety) Act 1998, Part 5, Division 3 'Orders controlling food production'.

Re-opening Criteria

A shellfish growing area, previously closed for harvesting based on toxin data, can only re-open when the "State Coordinator" has data to demonstrate that the toxin concentration in shellfish meat is below the relevant concentration specified in the above closure criteria. Toxin concentrations in shellfish from adjacent areas must also be considered prior to the re-opening. However, no guidelines are given as to the number of favourable toxin results that must be obtained, and the minimum period of time over which the shellfish samples can be collected, before a re-opening may occur.

The re-opening of a growing area previously closed as a precautionary measure can only occur when the State Coordinator has sufficient evidence to show that shellfish harvesting can safely proceed.

No re-opening criteria are specified for DSP and NSP toxins. In addition, there are no criteria based on the absence or reduction in abundance of the causative toxic algal species to cell concentrations below a prescribed abundance, and no criteria based on the absence of any shellfish poisoning reported since the date of the first 'clear' biotoxin sample.

Program Administration

The Department of Land and Water Conservation (DLWC) is the agency currently responsible for freshwater and marine algal bloom management in NSW. DLWC has established eight 'Regional Algal Coordination Committees' (RACCs) that have broad representation, to coordinate appropriate responses to any algal bloom. The RACCs follow guidelines provided by the 'State Algal Coordinating Committee' (SACC), a body established in the early 1990s to oversee the 'State Algal Management Strategy' concerning blue-green algal blooms in freshwater systems. Each RACC has developed a 'Regional Algal Contingency Plan' for the coordination and management of all fresh water blooms in their region. Four RACCs cover the coastal regions within NSW: the North Coast; Hunter and Manning/Karuah/Great Lakes (served by same secretariat); and Metropolitan/South Coast Committees.

The main roles of the Regional Algal Coordinating Committees (RACCs) include: the development, coordination and implementation of algal bloom contingency strategies; coordination of regional

media relations and public information programs related to algae; the development, coordination and implementation of regional algae monitoring programs; the coordination and implementation of training in algal sampling and identification; and, identifying when an algal warning should be issued and which agency should issue statements concerning algal bloom warnings and clearances.

In February 2000 the Premiers Department of NSW announced that DLWC would also take responsibility for the coordination of responses to all marine algal blooms. Since this time the regional coastal committees have commenced the development of marine algal bloom contingency plans and the Metropolitan/South Coast RACC has produced a draft 'Marine and Estuarine Algal Contingency Plan'. However, the roles and responsibilities of the various agencies (EPA, Safe Food Production NSW, NSW Fisheries, NSW Health, etc) involved in responding to marine and estuarine algal blooms have not yet been finalised. Both the DLWC and Safe Food Production NSW report to the Minister of Agriculture.

The NSW SQAP is a member of, or communicates closely with, the four coastal Committees. Furthermore, the Program Manager of the NSW SQAP is responsible for the management of marine algal blooms that have the potential to impact farmed shellfish. However, no routine marine algal monitoring is currently conducted or coordinated by any of the coastal Committees in NSW. Consequently, in the event of a marine algal bloom the work conducted by the relevant RACC (and the NSW SQAP) is largely reactionary.

As previously stated, the NSW SQAP was transferred from NSW Fisheries to Safe Food Production NSW in 1999. The Program currently operates under amended Fisheries legislation that provides for the Minister for Agriculture to have responsibility for the Program with the concurrence of the Minister for Fisheries. This is a short-term measure and will only continue until the Seafood Safety Scheme Regulation is enacted under the Food Production (Safety) Act 1998.

It is intended that the proposed Seafood Safety Scheme Regulation will incorporate the NSW SQAP, which will continue a shellfish safety program under the new regulatory framework. Where NSW Fisheries legislation limited the NSW SQAP area of operation to farmed bivalves in NSW, the new Regulation will incorporate all bivalve shellfish (both cultivated and wild harvest). The Seafood Safety Scheme will apply food safety risk management principles to all species of seafood harvested for human consumption in NSW, adopting requirements specific to the species concerned and the harvest locality and environment. In summary, the Seafood Safety Scheme Regulation will have the power to impose necessary food safety measures, including biotoxin management, on all sectors of the seafood industry.

In the interim, the Marine Algal Biotoxin Management/ Reaction Plan is the key document setting out the roles and responsibilities of the NSW SQAP and Safe Food Production NSW concerning toxic marine algal blooms. Major factors discussed include: communication strategy; contingency and notification procedures; closure and re-opening protocols; product recall procedures; data management; and laboratory support.

The Plan states that the absence of algal and biotoxin monitoring "does not absolve shellfish industries of the responsibility to ensure that product meets legal requirements and that biotoxin incidences are effectively managed". However, how this may be achieved without the benefit of necessary routine monitoring is not addressed.

Internal Review

No specific date is provided concerning the first formal review of the Marine Algal Biotoxin Contingency/Management Plan, although it is planned to develop "future strategies" based on the findings of the current Hawkesbury River/ Berowra Creek research project in New South Wales and on the recommendations of the present 'Australian National Biotoxin Strategy'. A comprehensive review of the Plan will not occur until the Seafood Safety Scheme Regulation is implemented about mid 2001.

Due to the current complexities concerning biotoxin management arrangements in NSW, Safe Food NSW provided a very complete and thorough response to the State biotoxin questionnaire. The extensive comments and attached documents were greatly appreciated.

Biotoxin Management Plans for the Pipi Industry

Mandatory biotoxin surveillance by the NSW pipi industry was introduced following the two outbreaks of (unconfirmed) diarrhetic shellfish poisoning associated with the consumption of pipis containing PTX2 and PTX2sa from Ballina in late 1997 and from Anna Bay/ Stockton Beach (Newcastle) in early 1998. The Director of Fisheries announced that from 1 December 1998 the commercial harvesting of bivalve molluscs from ocean beaches and pipis from all locations could only continue if commercial fishers operated under a biotoxin management plan that met an agreed standard. No distinction was made between product sold for human consumption or bait. Bait pipis (sold frozen) also had to comply with the food safety requirements.

The biotoxin management plans were prepared by industry in line with established best practice, such as that documented in the 'Operations Manual' of the Australian Shellfish Sanitation Control Program. Written procedures were required that addressed the following ten elements: closure criteria; re-opening criteria; maintenance of records documenting the basis of all decisions; internal auditing and the implementation of corrective actions; bi-annual third-party auditing of the plan; forwarding of third-party auditor reports to NSW Fisheries; product labelling to enable product tracking and recall; response capability given a food poisoning event; member training to implement the plan; and the labelling and separation of bait pipis from pipis collected for human consumption. Ongoing routine monitoring of algae and/or biotoxins in pipis was identified as the key issue.

The biotoxin management plans could be developed and operated by individual fishers or groups of fishers acting through an association or fishermen's co-operative. The use of specialist consultants for plan development was recommended but was not a formal requirement. However, all plans had to be initially verified by an independent food safety professional. Plan development and implementation also had to be funded entirely by industry.

Later, in February 1999, NSW Fisheries prohibited the recreational harvesting of pipis from ocean beaches for human consumption. Pipis can still be collected and used for bait in the immediate vicinity of the beach, but cannot be taken beyond 50 metres of the high water mark. This latter control was introduced as a precautionary measure to prevent the excessive harvesting of pipis by the recreational sector and to prevent further food poisoning outbreaks due to marine biotoxin contamination.

The need for a biotoxin management plan currently does not apply to the commercial gathering of bivalve shellfish (e.g. cockles and mussels) from estuaries or marine embayments. It applies only to bivalves gathered from ocean beaches. In practice, the mandatory requirement has been limited to the pipi industry. Pipis are restricted in their distribution to high-energy ocean beaches.

There are currently five biotoxin management plans (and 43 pipi fishers) on a NSW Fisheries biotoxin plan register. Seafood safety consultants developed two of the plans, while the fishers themselves developed the other three plans. Food safety auditors have audited all five plans at least once.

No pipi biotoxin management plans were forwarded by NSW Fisheries for formal review as they are considered to be the property of the relevant pipi associations. However, a pre-existing knowledge of some plans, together with recent discussions with several plan coordinators, has provided some insight as to the key elements of each plan. All rely on weekly phytoplankton monitoring, of varying style and intensity, and on the biotoxin testing of pipi meat when cell concentrations of potentially toxic algal species exceed specified 'action levels' (mostly based on New Zealand criteria).

All five pipi groups have been affected by beach closures. Harvesting suspensions have been necessary due to the presence of *Dinophysis acuminata* and/or *D. caudata*, or blooms of *Pseudo-nitzschia* spp. Three food recalls involving NSW pipis were reported by the Australian New Zealand Food Authority (ANZFA) in the period from August 1999 to May 2000. Two recalls of pipis harvested from Yagan Beach / Seal Rocks were necessary due to high levels of potentially toxic algae (*Pseudo-nitzschia* spp.), and one recall of pipis from South Ballina Beach as a precautionary measure due to the detection of low concentrations of pectenotoxins. No further cases of illness associated with the consumption of NSW pipis have been reported since late 1997 and early 1998.

5.2.1 Key Strengths of NSW SQAP Biotoxin Management Arrangements

(i) A Marine Algal Biotoxin Contingency/Management Plan has been developed and is implemented for oyster and mussel culture industries to provide a response capability when toxic algal blooms are reported by the shellfish industry or government agencies.

(ii) Existing strong legislative powers allow the NSW SQAP Program Manager to close oyster and mussel culture areas if biotoxins detected or, as a precautionary measure, when potentially toxic algal blooms or unusual environmental conditions observed.

(iii) Marine algal bloom contingency plans currently being developed by coastal 'Regional Algal Coordination Committees' since Premiers Department 2000 directive that Department of Land and Water Conservation assume responsibility for the coordination of responses to all marine algal blooms.

(iv) Biotoxin management plans, incorporating algal and biotoxin monitoring, developed and implemented by pipi industry to ensure safety of pipis sold both for food and bait.

(v) Recreational harvesting of pipis for human consumption prohibited in an attempt to prevent future shellfish poisoning outbreaks.

(vi) Proposed 'Seafood Safety Scheme Regulation', when enacted, will provide the power for Safe Food Production NSW to impose necessary food safety measures, including biotoxin management, on *all* sectors of the seafood industry.

5.2.2 Key Weaknesses of NSW SQAP Biotoxin Management Arrangements

(i) *No recurrent and contingency funding is available from government to run a satisfactory biotoxin monitoring program.*

The lack of clearly defined roles and responsibilities of the oyster and mussel culture industries, vis-à-vis Government, appears to be the major stumbling block preventing the initiation of routine phytoplankton and biotoxin monitoring that is urgently needed to ensure the safety of NSW commercial shellfish. It is argued in the Biotoxin Management/Reaction

Plan that "a comprehensive and routine monitoring program may well be beyond the capacity of industry to provide", given the number of estuaries involved and the level of funding and infrastructure required. Furthermore, the number of other beneficiaries of a monitoring program, and general public health considerations related to the recreational use of estuaries, supports the view that funding "should not necessarily be the sole responsibility of industry". The 'NSW Marine Algal Biotoxin Committee' earlier estimated, in their November 1995 report on 'The Management of Marine Algal Issues in NSW', that funding in excess of $1million would be required in the first three years of operation. Considerably more would be required today. The current development of a 'Marine and Estuarine Algal Contingency Plan' by the Metropolitan/ South Coast RACC, provides a unique opportunity to finalise the roles and responsibilities of the shellfish industry and government agencies, and to determine the appropriate level of funding required to be contributed by each agency.

(ii) *Inadequate public health protection from potential biotoxin contamination of commercially or recreationally harvested wildstock shellfish resources.*

The Marine Algal Biotoxin Contingency/Management Plan only concerns the safety of commercially cultured oysters and mussels grown in designated aquaculture areas. No routine phytoplankton monitoring occurs in wild harvest shellfish areas (except at beaches where pipis are commercially harvested), and no routine biotoxin testing is conducted on commercial wild harvest shellfish such as scallops, native flat oysters, blue mussels and cockles. A thorough biotoxin risk assessment should be conducted for all commercial wild harvest shellfish, which should all be included in a biotoxin management program to provide necessary public health protection. The degree of risk will depend in part on exactly what edible tissues are consumed. In the case of scallops, for example, whole tissue or 'roe-on' meat poses a higher risk than adductor muscle meat.

(iii) *Key elements and considerable detail concerning biotoxin management are lacking in the current Marine Algal Biotoxin Contingency/Management Plan.*

Consideration should be given to all of the components outlined in the "Suggested contingency plan for control of marine biotoxins" contained in Appendix VI of the 'Operations Manual of the Australian Shellfish Sanitation Control Program'. One key component missing is an adequate early warning system incorporating a routine monitoring program (discussed elsewhere). Procedures to define the severity of a toxic event, including the additional resources required to promptly expand the sampling and testing program, should also be documented. Notification protocols are provided but no actual contact details are listed for any individual or agency. Detailed product recall procedures and phytoplankton and biotoxin analytical methods are also required. Ideally, the Plan should be a stand-alone document that contains all necessary information to enable appropriate biotoxin contingency arrangements to proceed smoothly even in the absence of the Program Manager.

(iv) *Absence of routine phytoplankton monitoring in some oyster and mussel culture areas to provide necessary early warning of the development of toxic algal blooms.*

As a number of toxic algal species are known to occur in NSW waters, and toxic algal blooms and cases of shellfish poisoning have already been reported in the State, phytoplankton monitoring is necessary to provide adequate public health protection for all consumers of NSW cultured shellfish. Phytoplankton monitoring enables all potentially toxic species to be detected when they first appear and warns of the potential for marine biotoxins to be detected in shellfish. Frequent phytoplankton analysis reveals whether a toxic species is increasing or decreasing in abundance and indicates the type of biotoxin analysis required at the time (reducing the need for multiple biotoxin tests. It also provides results in a timelier manner and is cheaper than shellfish testing. If phytoplankton monitoring is conducted, 'action levels' can be specified for individual toxic algal species to initiate relevant and timely toxin testing, or to close a shellfish growing area pending the

results of toxin analyses. The reporting of algal blooms and unusual environmental phenomena by industry and relevant government agencies, as occurs under present arrangements, is useful but is no substitute for regular and frequent phytoplankton and biotoxin monitoring.

(v) *Biotoxin risk assessments have not been conducted for most of the NSW estuaries used for commercial shellfish production.*

There is a paucity of data concerning the distribution of toxic marine algae in NSW estuaries. However, toxic algal cells and cysts have been detected in several estuaries and a number of toxic bloom events have been documented. Due to the current lack of funding for routine monitoring, it has been argued that programs should initially be developed for these "hotspots" or high-risk areas. However, routine phytoplankton monitoring is urgently required in *all* estuaries used for commercial shellfish production. Consequently, a biotoxin risk assessment should be undertaken for all relevant estuaries. The risk assessment should ideally involve an initial survey of viable toxic algal cysts in estuarine sediments (where sediments suitable), followed by the collection and examination of phytoplankton samples throughout the year. Monthly sampling for 3 or more years, to allow for possible substantial interannual variation in algal composition, may be sufficient for those estuaries where toxic algae are not known to occur. However, the frequency and intensity of monitoring would have to be increased if any toxic algal species were detected. In this way, the phytoplankton component of the risk assessment should provide necessary public health protection from marine toxic algae in the early years of the biotoxin surveillance program.

(vi) *Additional closure criteria, in addition to that for PSP and ASP toxins, are necessary to ensure adequate public health protection.*

Closure criteria based on PSP and ASP toxin concentrations are provided in the current Marine Algal Biotoxin Contingency/Management Plan. However, regulatory limits for all four main toxin types are now included in the Australian New Zealand 'Food Standards Code', so closure criteria should be provided for DSP and NSP toxins. Furthermore, the existing criteria for PSP and ASP toxins should be expressed in terms of 'saxitoxin equivalent' and 'domoic acid equivalent' respectively, as worded in the Code. Additional closure criteria should be added based on the cell concentration of toxic algal species exceeding specified 'action levels', levels prescribed to initiate a closure pending the results of toxin testing of shellfish meat. There are also no criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

(vii) *Complementary re-opening criteria matching the additional closure criteria are required, together with guidelines for their application.*

In regard to the present re-opening criteria for the potentially lethal PSP and ASP toxins, it is necessary to state that toxin concentrations should be less than the relevant regulatory limit in three consecutive samples collected over a minimum period of 14 days. Criteria to re-open a shellfish growing area previously closed due to contamination by DSP or NSP toxins should be added. For all toxins, concentrations should be below the relevant regulatory limit in three consecutive samples collected over a minimum period of 14 days. The concentration of the toxic algal species responsible for the closure should also be clearly decreasing and remain below the prescribed 'action level' for that species. Lastly, no cases of human illness, fitting the accepted case definitions for PSP, ASP, DSP or NSP, should have resulted from the consumption of any shellfish harvested from within or adjacent to the closed area since the date of the first 'clearance' sample. Words such as 'clearance' or 'negative' need to be defined.

(viii) *Annual reviews of the Marine Algal Biotoxin Contingency/Management Plan are required.*

Annual reviews are needed to re-assess phytoplankton and biotoxin monitoring and other early-warning strategies, and to evaluate the efficacy of biotoxin management controls given a further 12 months' data and experience. Regular reviews are also necessary to ensure that

the Plan always reflects current operating procedures; the current Plan is somewhat outdated and needs urgent review.

(ix) *No scientific or funding support provided by government to pipi industry.*
Due to the two outbreaks of DSP resulting from the consumption of NSW pipis, pipi industry members were required to quickly develop, implement and fund a biotoxin management plan, including routine biotoxin monitoring, for each beach where commercial harvesting was to be continued. However, despite the uncertainty concerning the toxin(s) responsible for the poisoning, and the lack of a commercial analytical method to determine the concentration of the relevant toxin(s) in pipi meat, no scientific or financial support was provided by NSW Fisheries to the pipi industry. Five biotoxin management plans, of variable quality, were consequently developed by groups of commercial pipi fishers. More assistance, including both scientific and funding support, is required from government to ensure that all plans are operating effectively. An evaluation of the efficacy of the current third party auditing system is also recommended.

(x) *The relevance of New Zealand "action levels" for toxic algal species present in local waters has not been assessed.*
One or two 'action levels' (specified cell concentrations) have been listed for some toxic or potentially toxic algal species in several pipi biotoxin management plans. Cell concentrations exceeding the action levels immediately trigger relevant biotoxin testing of pipi meat. Harvesting may continue when the first action level is exceeded but must be suspended, pending the results of toxin analyses, when the second level is exceeded. In the absence of an Australian or NSW list of action levels, New Zealand cell concentrations have been used. This may be acceptable in the first instance, but their relevance should be assessed based on experience gained in local waters. Strict adherence to the New Zealand levels has already resulted in two unnecessary food recalls involving pipis harvested from the Yagan Beach/ Seal Rocks area of NSW.

5.3 Northern Territory Department of Primary Industry and Fisheries

Responsible Agency
Biotoxin management matters fall within the portfolio of the Deputy Director, Fisheries Division, Department of Primary Industry and Fisheries.
Current Deputy Director: Rex Pyne.

Shellfish Quality Assurance and Biotoxin Management
There is currently no shellfish quality assurance program (including biotoxin monitoring) in operation in the Northern Territory, and no 'Memorandum of Understanding' has been developed with the Australian Quarantine and Inspection Service (AQIS) in order to allow the export of Territory bivalve shellfish. However, the Northern Territory is represented at the biannual meetings of the 'Australian Shellfish Quality Assurance Advisory Committee' (ASQAAC).

No biotoxin testing of bivalve shellfish has been conducted in the Northern Territory to date. Consequently, no biotoxin related documents were available for review.

Several toxin-producing algal species with the potential to cause PSP, ASP or DSP are present in Northern Territory waters. The toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* has caused major PSP problems in the neighbouring tropical Indo-West Pacific including Brunei, Indonesia, Palau, Papua New Guinea, the Philippines, Sabah and the Solomon Islands (Hallegraeff & MacLean 1989). Benthic resting cysts of this species have been discovered in the Port of Darwin, although no motile planktonic cells have been observed in Australian waters (McMinn quoted in Hallegraeff 1992). Potentially toxic *Pseudo-nitzschia* species and *Dinophysis* species are also present in northern Australian waters. No list of potentially toxic algal species is currently available within the Fisheries Division.

The Fisheries Division is unaware of any cases of shellfish poisoning having been reported in the Northern Territory. The power to suspend harvesting and close shellfish growing areas due to biotoxin contamination is provided in the NT Fisheries Act.

Bivalve Shellfish Resources and Harvesting Practices
A very diverse range of bivalve molluscs is present in Northern Territory waters. Both these and gastropod molluscs are harvested by Aboriginal people for subsistence purposes and by others for recreational purposes. Shellfish are an important protein source for Aboriginal people, who have access to 84% of the Northern Territory coastline for food gathering activities. There is no limitation concerning recreational harvesting other than the harvesting bag limits.

Commercial shellfish harvesting or culture is very low key in the Northern Territory at present and the current situation is not expected to change significantly in the short to medium term.

Mud scallops (*Amusium pleuronectes*) are harvested and landed for human consumption as a by-catch by the Northern Prawn Fishing (NPF) fleet. About six tonne of scallops are sold on domestic markets mainly in the shell. The percentage of whole tissue versus 'roe-on' or 'roe-off' meat consumed is not known.

Pearl oysters (*Pinctada* species) are cultivated mainly for pearls, although the adductor muscle is sometimes sold for human consumption. Exports of pearl oyster meat took place up to fairly recent times but are currently banned by AQIS.

Some limited experimental work is being conducted on the cultivation of the edible black-lip oyster *Pinctada margaritifera*. If successful, the oyster-culture industry will have to fully comply with the requirements of the Australian Shellfish Quality Assurance Program (ASQAP). Biotoxin surveillance would therefore be necessary.

5.3.1 Key Biotoxin Management Weaknesses

(i) No assessment made of the potential risk to public health caused by the presence of toxic algae in Northern Territory waters.
(ii) No biotoxin management plan available to guide emergency response in event of a toxic algal bloom or shellfish-poisoning outbreak.
(iii) No funding available for phytoplankton or biotoxin monitoring or to conduct relevant biotoxin risk assessments.
(iv) Lack of specialist biotoxin management expertise available to NT Fisheries.
(v) No public health protection from potential biotoxin contamination of commercially harvested scallops and commercially cultured pearl oysters.
(vi) No public health protection from potential biotoxin contamination of recreationally harvested wildstock shellfish resources.

5.4 Queensland Shellfish Water Assurance Monitoring Program (QSWAMP)

Date of Latest Plan
A 1998 'Biotoxin Contingency Plan for Moreton Bay Oyster Industry' was made available for evaluation.

Responsible Agency
Queensland Fisheries Service, Queensland Department of Primary Industries (QDPI).
Current Program Manager: Mr Kerrod Beattie.

Brief History of Biotoxin Surveillance
The Queensland Shellfish Water Assurance Monitoring Program (QSWAMP) commenced in 1993 to ensure the safety of Sydney rock oysters cultured in Moreton Bay, Brisbane. Three large growing areas were classified: Area A situated along the southern foreshore of Moreton Island (currently consisting of 24 individual licensed areas), and Areas B & C situated along the northern foreshore of North Stradbroke Island north and south of Dunwich respectively (combined total of 68 licensed areas).

To date biotoxin monitoring has been limited to the occasional testing of oysters for both PSP and ASP toxins by mouse bioassay and HPLC analysis respectively. One oyster sample from each of the three main growing areas is tested on each occasion. The first analyses were conducted in October 1993 and 21 sets of analyses have been conducted to the present time. Samples are especially taken immediately before the start of harvesting each year and before harvesting for exports; only oysters from Moreton Bay are exported. No PSP toxins or domoic acid have been detected.

No routine phytoplankton monitoring has been conducted in Moreton Bay as a biotoxin monitoring tool, although the Queensland EPA and University of Queensland have conducted some phytoplankton studies in the Bay.

Three additional shellfish growing areas for Sydney rock oysters (Pumicestone Passage, South Stradbroke Island and Southport/Gold Coast) are presently being classified as part of the QSWAMP. These three areas contain a combined total of 68 individual licensed areas. Biotoxin monitoring will be conducted at these localities when the classification is completed.

By far the largest bivalve shellfish industry in Queensland is the scallop industry, which harvests both saucer and mud scallops. Exports of adductor muscle, 'roe-on' or whole tissue of both species occurred up to fairly recent times, but AQIS now only permit the export of saucer scallop flesh (adductor muscle and small quantities of 'roe-on' meat). Scallop viscera appears to be at much higher risk from marine biotoxin contamination than adductor muscle meat (although this needs to be confirmed for Australian scallop species. About 90% of saucer scallop meat is exported and the other 10% sold domestically. No biotoxin testing of saucer or mud scallops has been undertaken as part of the QSWAMP.

Other bivalve shellfish resources, which are harvested either commercially and/or recreationally, include milky and black-lip oysters, pearl oysters, pipis and cockles (see details of bivalve resources and their distribution below). Biotoxin monitoring is not conducted for these shellfish.

Biotoxin monitoring in Queensland is funded 50% by the Queensland Fisheries Service and 50% by the oyster industry operating in Moreton Bay. The direct cost of biotoxin testing in 2000/2001 is about $7,000. Government is also responsible for ongoing biotoxin management, reporting etc.

No cases of illness have resulted from the consumption of Queensland shellfish contaminated with biotoxins to date. However, toxic and potentially toxic algal species have been detected in Queensland waters, and therefore there is a very real risk of marine biotoxin contamination of shellfish.

Bivalve Shellfish Resources

- Sydney rock oyster (*Saccostrea glomerata*)
- Milky oyster (*Saccostrea amasa*)
- Black-lip oyster (*Pinctada margaritifera*)
- Ballots saucer scallop (*Amusium balloti*)
- Mud scallop (*Amusium pleuronectes*)
- Giant clams (*Tridacna maxima, T. squamosa* and *Hippopus hippopus*)
- Pearl oyster (*Pinctada maxima*)
- Penguin wing oyster (*Pteria penguin*)
- Pipi (*Plebidonax deltoides*)
- Cockle (*Anadara trapezia*)

Sydney rock oysters are commercially cultured from natural spatfall, although most spat are imported from NSW. Both cultured and wild harvest oysters are harvested mainly from the NSW border to Bundaberg. Some spatfall occurs as far north as Townsville, but these spat are unlikely to survive the warmer summer temperatures in northern Queensland due to QX disease. The commercial oyster areas occur in both coastal marine and estuarine systems.

Milky oysters are grown from natural spat on rocky foreshores and islands ranging from Bundaberg to Cape York, as are black-lip oysters (young pearl oysters) that are distributed from mid North Queensland to Cape York. There are currently 91 licensed areas for the commercial harvesting of either milky or black-lip oysters; each individual area consists of about 600 m of foreshore. Black-lip oysters are "cultured" both for pearls and whole meat. The recreational harvesting of oysters is permitted outside of commercial growing areas, but they must be eaten on the beach where collected.

The saucer scallop ranges in distribution from about 18°S to 35°S, although the fishery mainly occurs between 20°S and 26°S. Trawling occurs in water depths of 20-50 m. Mud oysters have a wide but somewhat discontinuous distribution around the northern coastline of Australia, and occur down to about 22°S on the Queensland coastline (17°S-20°S for fishery). The mud scallop is a more inshore species found in depths of 5-15 m. Scallop trawling is not permitted in the Great Barrier Reef Marine Park Authority's (GBRMPA) General use B, National Park A & B and Preservation Zones.

Giant clams, which are protected species, are widely distributed in reef areas. *Tridacna maxima* is found along the entire Queensland coastline, while *T. squamosa* and *Hippopus hippopus* range from Queensland to northern Western Australia. The latter species occurs on reef flats and is hence very susceptible to illegal harvesting. There are five areas from Brisbane to Cairns where some research is being conducted on giant clam culture. Unauthorised exports of Queensland clam meat (the adductor muscle) have occurred via the Northern Territory in fairly recent times.

In addition to the black-lip oyster, the pearl oysters *Pinctada maxima* and *Pteria penguin* are commercially cultured and harvested from northern Queensland waters for pearls and oyster meat (the adductor muscle).

Pipis are found on southeast Queensland surf beaches with a distribution from the Gold Coast to North Stradbroke Island, Moreton Island and north to Fraser Island. There are currently no commercial licenses but an application for wild harvesting is being considered.

Cockles, found on mud flats along the entire Queensland coastline, are subject to recreational harvesting. However, there are some closed areas and a bag limit applies in open areas.

Phytoplankton and Biotoxin Monitoring

Currently no phytoplankton monitoring is conducted in Queensland shellfish growing areas to provide an early warning of potentially toxic or harmful algal blooms. Biotoxin monitoring is conducted but not routinely and only within the three main shellfish growing areas for Sydney rock oysters in Moreton Bay. Current practice involves the testing of a single oyster sample from each of the three areas for PSP toxins and domoic acid 4-6 times per year. A priority is given to those periods immediately before the start of seasonal harvesting and prior to export shipments. The oysters are at their prime from July to September, after which their condition deteriorates due to QX disease as the water temperature increases.

There is provision in the Biotoxin Contingency Plan to substantially increase the frequency and spatial coverage of the biotoxin testing if any toxin was detected during normal testing or if the development of a toxic algal bloom was reported by industry or other associated agencies.

Queensland Fisheries Service officers conduct all the field sampling.

Closure and Re-opening Criteria

Closure Criteria

Shellfish growing areas will immediately be closed for harvesting when either of the following biotoxin criteria is satisfied:

- The concentration of paralytic shellfish poison (PSP) exceeds or is equal to 0.8 mg/kg of the edible portion of raw shellfish.
- The concentration of domoic acid exceeds or is equal to 20 mg/kg of the edible portion of raw shellfish.

These criteria are the same as those specified in the current ‘Operations Manual’ of the Australian Shellfish Quality Assurance Program (ASQAP).

No criteria are provided for DSP or NSP toxins. As routine phytoplankton monitoring is not conducted there are also no closure criteria based on potentially toxic algal species exceeding a prescribed abundance, pending the results of toxin testing of shellfish meat.

It is indicated in the Plan that additional shellfish sampling and biotoxin testing would occur if the Queensland Department of Health (QDOH) receives reports of shellfish poisoning. However, there is no specific closure criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

The power to suspend shellfish harvesting and close shellfish growing areas is provided under Section 96 of the *Fisheries Act 1994*. If the Executive Director (Fisheries) is of the opinion that

urgent action is required he may issue an "emergency disease declaration". This declaration may make provision for the matters the chief executive considers necessary or desirable for the management, control and elimination of the declared disease. "Disease" in this context means: (a) a disease, parasite, pest, plant or other thing (the "disease") that has, or may have, the effect (directly or indirectly) of killing or causing illness in fisheries resources, or in humans or animals that eat fisheries resources infected with or containing the disease; (b) a chemical or antibiotic residue.

Re-opening Criteria
Shellfish growing areas will be re-opened for shellfish harvesting when three (3) consecutive shellfish samples, taken from the same site, over a minimum period of 14 days, satisfy the following toxin criteria:

- The concentration of paralytic shellfish poison (PSP) does not exceed or equal 0.8 mg/kg of the edible portion of raw shellfish.
- The concentration of domoic acid does not exceed or equal 20 mg/kg of the edible portion of raw shellfish.

No re-opening criteria are specified for DSP and NSP toxins. Furthermore, no criteria are provided based on the absence or reduction in abundance of the causative toxic algal species to cell concentrations below a prescribed abundance, or criteria based on the absence of any shellfish poisoning reported since the date of the first "clear" biotoxin sample.

Program Administration
QDPI and the Queensland Department of Environment and Heritage (QDEH) have signed a joint Memorandum of Understanding (MOU) with the Australian Quarantine Inspection Service (AQIS) concerning the sanitary control (including marine biotoxin control) of fresh and frozen molluscan shellfish intended for exportation from Queensland. An Inter-Agency Agreement has also been developed between QDPI and the Queensland Department of Health (QDOH). These formalised agreements define the respective roles and responsibilities of each agency concerning the Moreton Bay oyster industry and the Biotoxin Contingency Plan.

The Moreton Bay Biotoxin Contingency Plan closely follows the format of the 'Suggested Contingency Plan for Control of Marine Biotoxins' documented in Appendix VI of the 'Operations Manual of the Australian Shellfish Sanitation Control Program'. All key requirements are satisfied with regard to the Moreton Bay oyster industry with one significant exception. No routine phytoplankton or biotoxin monitoring is conducted to provide sufficient early warning of potentially unsafe conditions. QDPI currently rely on limited biotoxin testing and notifications of bird or fish kills, abnormal shellfish behaviour and water discolouration (caused by algal blooms) as their early warning system. Reports of any shellfish poisoning would also be provided by QDOH if any outbreak occurred.

The QDPI are responsible for the development and implementation of the Biotoxin Contingency Plan in relation to oyster growing areas. The Executive Director (Fisheries), following consultation with the QDOH and Queensland Oyster Growers Association (QOGA), makes all closure and re-opening decisions. Procedures and notifications concerning the closure and re-opening of an oyster growing area are detailed in the current Plan. It is the duty of QDPI to notify all relevant agencies, the QOGA and individual oyster growers. Draft closure and re-opening notices and draft media releases have been prepared.

Once oyster shellstock has been harvested it is deemed to be a "food" and hence comes under the responsibility of the QDOH. The Department has the power under the *Food Act 1981* to recall and/or embargo shellstock or shellfish meat destined for commercial sale.

Oyster farmers are required to notify QDPI if they observe any disease, mortality or unusual environmental phenomena (Section 100, *Fisheries Act 1994*). These notifications may help to provide some early warning capability in some situations. Oyster growers must cease harvesting whenever a closure notice is issued, and they are not permitted to re-commence harvesting in a previously closed growing area until they receive a formal letter from QDPI advising them of a re-opening.

Officers of the Queensland Boating and Fisheries Patrol will perform necessary surveillance and inspection services to prevent the harvesting of any shellfish from a closed oyster area. QSWAMP officers collecting samples for biotoxin testing provide additional surveillance support. Under the *Food Hygiene Regulations 1989*, Local Governments must ensure that oyster shellstock from closed areas are not processed in registered or non-registered premises.

Internal Review

Although it is planned to conduct an annual reappraisal of the classification of each oyster growing area in Moreton Bay, there are no stated plans concerning the review of the Biotoxin Contingency Plan.

5.4.1 Key Strengths of QSWAMP Biotoxin Contingency Plan

(i) The risk of shellfish poisoning appears to be comparatively low, vis-à-vis most other States. No suspensions of shellfish harvesting have been necessary in Queensland's marine aquaculture areas to date.

(ii) QDPI and QDEH jointly responsible for administration of WASQAP (signed MOU with AQIS), with an additional Inter-Agency Agreement developed between QDPI and QDOH concerning biotoxin contingency arrangements.

(iii) Well-developed arrangements to define the severity of the problem and to respond effectively to minimise illness, in the event of a toxic algal bloom.

5.4.2 Key Weaknesses of QSWAMP Biotoxin Contingency Plan

(i) *Insufficient annual funding is available to the QSWAMP Manager to run a satisfactory biotoxin management program.*

The budget of only $7,000 for biotoxin surveillance in 2000/01 is grossly inadequate. A substantially increased budget is necessary to conduct routine phytoplankton monitoring, and relevant biotoxin testing, to ensure the safety of all commercially cultured or wild harvested bivalve shellfish in Queensland marine waters. Biotoxin surveillance is necessary to avoid possible food poisoning outbreaks and to protect the viability of Queensland's commercial shellfish industries. The funding allocation for 2001/02 is currently unknown.

(ii) *No contingency funding is available to the QSWAMP Manager to investigate toxic bloom events as they occur.*

There is no contingency funding allocation in the QSWAMP budget to enable the Manager to conduct urgent unplanned phytoplankton monitoring and biotoxin testing during the development of a toxic algal bloom. Additional funding is needed to increase the frequency and spatial coverage of phytoplankton monitoring and to conduct extra biotoxin tests to define the severity and size of the bloom and to prevent the harvesting of contaminated shellfish. To be able to investigate any potential threat in a timely manner appropriate funding should be available prior to the event.

(iii) *Lack of specialist biotoxin management support available to the QSWAMP Manager.*
Additional biotoxin management expertise should be made available to the QSWAMP Manager to enable him to conduct necessary biotoxin risk assessments and to develop appropriate biotoxin surveillance strategies for all commercial aquaculture and wild harvest shellfish industries.

(iv) *Inadequate public health protection from potential biotoxin contamination of commercially or recreationally harvested wildstock shellfish resources is in place.*
Current biotoxin surveillance conducted by the QSWAMP only concerns the safety of commercially cultured Sydney rock oysters grown in 92 of 160 licensed areas. No routine phytoplankton monitoring or biotoxin testing on commercial wild harvest shellfish such as scallops, clams, pipis, cockles and wildstock milky, black-lip and pearl oysters occurs in wild harvest shellfish areas. A thorough biotoxin risk assessment should be conducted for all commercial wild harvest shellfish, which should be included in a biotoxin management program to provide necessary public health protection. The degree of risk will depend in part on exactly what edible tissues are consumed. In the case of scallops, for example, whole tissue or 'roe-on' meat poses a higher risk than adductor muscle meat.

(v) *Biotoxin monitoring conducted at existing oyster growing areas in Moreton Bay is inadequate to detect the presence of biotoxins in a timely manner, and no monitoring conducted at other oyster culture areas.*
At present biotoxin monitoring (for PSP and ASP toxins only) is conducted only 4-6 times per year at the three main oyster growing areas in Moreton Bay. Monitoring occurs mainly before the start of a harvesting season and sometimes before harvesting oysters for export. QDPI also receive notification of any abnormal environmental phenomenon occurring at a shellfish growing area, from industry or other relevant government agencies, to provide some additional advanced warning of potentially toxic algal blooms. These arrangements are inadequate to detect the presence of a toxic algal bloom or biotoxins in cultured oysters in a timely manner. It also raises the issue of a possible double standard concerning public health protection for consumers of oysters exported or sold on the domestic market. No monitoring is currently undertaken at oyster culture areas outside Moreton Bay.

(vi) *Absence of routine phytoplankton monitoring to provide necessary early warning of the development of toxic algal blooms.*
No phytoplankton monitoring is presently undertaken by the QSWAMP to provide an advanced warning of potentially toxic or harmful algal blooms. Yet phytoplankton monitoring has many advantages over biotoxin monitoring as a routine surveillance tool. Phytoplankton monitoring enables all potentially toxic species to be detected when they first appear and warns of the potential for marine biotoxins to be detected in shellfish. Frequent phytoplankton analysis reveals whether a toxic species is increasing or decreasing in abundance and indicates the type of biotoxin analysis required at the time (reducing the need for multiple biotoxin tests. It also provides results in a timelier manner than biotoxin testing and is cheaper than biotoxin analysis. If phytoplankton monitoring is conducted, 'action levels' can be specified for individual toxic algal species to initiate relevant and timely toxin testing, or to close a shellfish growing area pending the results of toxin analyses.

(vii) *Additional closure criteria, in addition to that for PSP and ASP toxins, are necessary to ensure adequate public health protection.*
Closure criteria based on PSP and ASP toxin concentrations are provided in the current Biotoxin Contingency Plan. However, regulatory limits for all four main toxin types are now included in the Australian New Zealand 'Food Standards Code', so closure criteria should be provided for DSP and NSP toxins. Furthermore, the existing criteria for PSP and ASP toxins should be expressed in terms of 'saxitoxin equivalent' and 'domoic acid equivalent' respectively, as worded in the Code. Additional closure criteria should be added based on the cell concentration of toxic algal species exceeding specified 'action levels',

levels prescribed to initiate a closure pending the results of toxin testing of shellfish meat. There are also no criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

(viii) *Complementary re-opening criteria matching the additional closure criteria are required.*
Criteria to re-open a shellfish growing area previously closed due to contamination by DSP or NSP toxins should be provided. The concentration of the toxic algal species responsible for the closure should also be clearly decreasing and remain below the prescribed 'action level' for that species. Lastly, no cases of human illness, fitting the accepted case definitions for PSP, ASP, DSP or NSP, should have resulted from the consumption of any shellfish harvested from within or adjacent to the closed area since the date of the first 'clearance' sample. Words such as 'clearance' or 'negative' need to be defined.

(ix) *Annual reviews of the Biotoxin Contingency Plan are required.*
Annual reviews are needed to re-assess the phytoplankton and biotoxin monitoring strategy and to evaluate the efficacy of biotoxin management controls given a further 12 months' data and information. Regular reviews are also necessary to ensure that the Plan always reflects current operating procedures.

5.5 South Australian Shellfish Quality Assurance Program (SASQAP)

Date of Latest Plan
April 2001.

Responsible Agency
Aquaculture South Australia, Primary Industries and Resources South Australia (PIRSA).
Current Program Manager: Mr. Ken Lee.

Brief History of Biotoxin Surveillance
The South Australian Shellfish Quality Assurance Program (SASQAP) began in early 1994 and was fully operational by January 1995. Six shellfish growing areas were classified in January 1996 and additional areas have been added since this time.

Biotoxin monitoring first started in late 1998. At this time there was no indication that toxic algal blooms were a potential risk factor in any of the commercial shellfish growing areas in the State. An earlier survey of toxic dinoflagellate cysts in sediment had been conducted at six shellfish growing areas (Hallegraeff & Andrijanic 1995), and no cysts of toxic species were detected. However, toxic blooms of the dinoflagellate *Alexandrium minutum* were known to occur regularly in the Port River and West Lakes (neither of which are commercial shellfish growing areas).

During the first year of phytoplankton monitoring toxic *Alexandrium* species capable of causing PSP were observed in several of the shellfish growing areas. Low concentrations of PSP toxins were found in razorfish (but not oysters) collected from commercial oyster growing areas along the coast of southern York Peninsula. The causative organism was an unidentified *Alexandrium* species, which appeared in December-January 1998/99 and again in January 2000. A public health alert was issued in January 2000, when growing area closures were necessary at Coobowie and Stansbury. A short 4-day closure (pending toxic test results) was also necessary at the Streaky Bay shellfish growing area in April 2000 due to the presence of *A. minutum*. In addition, a few cells of the toxic dinoflagellate *Gymnodinium catenatum* were recorded during routine monitoring at the Denial Bay shellfish growing area (Ceduna), and high concentrations of cysts of this species have been observed in sediments in Spencer Gulf. The presence of abundant cysts indicates a potential risk for the Port Lincoln and Louth Bay shellfish growing areas in particular.

Potentially toxic *Dinophysis* species have also been found to be widespread and occasionally relatively abundant along the South Australian coastline. DSP toxins (in particular, the pectenotoxins PTX2 and PTX2sa) have since been discovered in oysters and mussels at the time of blooms of *D. acuminata* and *D. caudata*. In October 1999 relatively high concentrations of *D. acuminata* were found at Nepean Bay, however no closure was considered necessary at the time as no biotoxins were detected in mouse bioassays. Area closures due to pectenotoxins have since been necessary at the Streaky Bay, Coffin Bay (Mt. Dutton Bay and Kellidie Bay), Port Lincoln (Bickers Island and Boston Bay) and the Nepean Bay (American River) shellfish growing areas. The longest closure to date was 7 months (February to August 2000) at the Mt Dutton Bay harvesting area. These closures were based on a conservative pectenotoxin closure criteria of 10 µg/100 g; more recent Australian and New Zealand data suggests that a criteria of 20 µg/100 g is more appropriate.

Another toxic dinoflagellate, *Karenia* cf *brevis* (=*Gymnodinium* cf *breve*), is frequently present in southern Spencer Gulf including Boston and Proper Bays. Brevetoxins (responsible for NSP) have been detected in algal samples containing K. *G.* cf *breve* but not in shellfish to date (shellfish analyses are very limited).

The approach taken by the SASQAP to minimise the risks associated with biotoxin contamination of commercial shellfish relies primarily on routine phytoplankton monitoring supported by relevant biotoxin analyses of shellfish meat when conditions are potentially unfavourable. The safety of cultured shellfish, mainly Pacific oysters and blue mussels, is the main focus. No phytoplankton monitoring is presently conducted in recreational harvesting areas outside the designated shellfish growing areas. However, there is an ongoing health warning advising the public not to harvest and consume bivalve shellfish from the Port River and West Lakes area because of possible contamination by algal toxins and other contaminants. No cases of shellfish poisoning have been reported in South Australia to date.

In addition to that contained in the Marine Biotoxin Management Plan, some relevant information on biotoxin management is also provided in the separate management plans developed for individual shellfish growing areas.

Biotoxin surveillance in South Australia is funded 50% by PIRSA and 50% by the farmed shellfish industry (oyster and mussel growers). The budget for FY 2000/2001 is $87,000 (salaries $40,000 and operating $47,000) excluding agency on-costs. No funding is contributed by the wild harvest shellfish industry or the recreational sector.

Bivalve Shellfish Resources

The following bivalve shellfish species are known to be present in South Australian waters:

- Pacific oyster (*Crassostrea gigas*)
- Native flat oyster (*Ostrea angasi*)
- Blue mussel (*Mytilus edulis*)
- Pipi (*Plebidonax deltoides*)
- Blood cockle (*Anadara trapezia*)
- Razorfish (*Pinna bicolor*)
- Queen scallop (*Equichlamys bifrons*)
- Southern scallop (*Pecten fumatus*)

Only Pacific oysters and blue mussels are cultured at this time (see shellfish growing areas below), although approval has been given for scallop farming at several sites including a 400 ha site at Wallaroo in Spencer Gulf. Most aquaculture licenses are 10 ha in size but growers generally start with only 1-3 ha.

Wild harvest shellfish include scallops, native flat oysters, pipis, blood cockles, and razorfish. Scallops are harvested from many areas in State. The majority of the commercial scallop catch in Coffin Bay, Spencer Gulf and Gulf of St. Vincent is queen scallops, while Southern scallops form a greater component on the west coast. Pipis are harvested commercially almost exclusively from a 21-km stretch of beach in the Coorong area. The pipis have traditionally been harvested for bait only, but industry is now keen to harvest for human consumption. Small numbers of blood cockles are harvested from the Port River and sold directly on the wharf.

Designated Shellfish Growing Areas

Current South Australia shellfish growing areas (including number of leases per area) are as follows:

Growing Areas	Harvesting Area	Number of leases
Denial Bay Shellfish Growing Area*	Denial Bay	16
	St. Peters	4
Smoky Bay Shellfish Growing Area*	Smoky Bay	13
	Waterwitch	7
Streaky Bay Shellfish Growing Area*	Blancheport	15
	The Hummocks	6
	Eba Island	1
Coffin Bay Shellfish Growing Area*	Coffin Bay	1
	Kellidie Bay	11
	Mt Dutton Bay	12
	Port Douglas	20
Port Lincoln Shellfish Growing Area*	Proper Bay	5
	Boston Bay	4
	Bickers Island	4
Louth Bay Shellfish Growing Area		5
Franklin Harbour Shellfish Growing Area*		22
Port Broughton Shellfish Growing Area		6
Nepean Bay Shellfish Growing Area	Eastern Cove	4
	Western Cove	13
	American River	3
Coobowie Shellfish Growing Area		7
Stansbury Shellfish Growing Area		9

*Areas surveyed for presence of toxic dinoflagellate cysts in sediment in 1994.

Phytoplankton and Biotoxin Monitoring

Phytoplankton Monitoring

Phytoplankton monitoring is the principal means of determining the potential for the contamination of shellfish by algal toxins in all shellfish growing areas. It provides an early warning of the presence and abundance of potentially toxic species and of the development of toxic algal blooms. All shellfish growing areas are covered in the plan.

Sampling sites are provided in individual management plans for each shellfish growing area. Multiple sites are monitored in large harvesting areas. The suitability and number of sampling sites in a particular area will be reviewed in light of experience.

Integrated water samples are routinely collected using a tube sampler from the surface to 1.5 to 4.0 metres of the water column depending on water depth at the sampling site. 500 ml sample bottles are filled from the integrated water samples. Water temperature, salinity, sampling time, tide, wind direction and speed, cloud cover and 72 hour rainfall are also recorded. Net tows are only used to concentrate cells for the purpose of species identification, culture initiation or to determine the biotoxin content of potentially toxic algae. All phytoplankton and shellfish samples are collected and dispatched by independent contractors.

The frequency of sampling varies according to the season as it is considered that the risk of toxic blooms is highest in summer. Samples are collected at least fortnightly in “summer” (October to April) and at least monthly in “winter” (May to September). However, the monitoring program is quite flexible and the number of sites and frequency of sampling are increased when the abundance of a particular toxic species exceeds the relevant category 1 ‘action level’ for that species (Table

10). Two categories of 'action levels' have been specified for the major toxic species (Table 10). If a cell concentration of a particular toxic species exceeds the 'category 1' concentration, shellfish must be collected and analysed for the relevant toxin type. However, if the cell concentration exceeds the higher 'category 2' concentration, all harvesting must cease pending the results of toxin analyses. A protocol for emergency sampling was also provided to the reviewers.

Phytoplankton identifications (toxic genera to species level except *Pseudo-nitzschia*) and cell counts are performed by SASQAP staff, although experienced external service providers are occasionally used for some routine sample analyses and their specialist taxonomic expertise. Methods of sample analysis were not provided.

Table 10. SASQAP Phytoplankton action levels

Phytoplankton species	Toxin type	Category 1 level Concentration to initiate flesh testing (cells per litre)	Category 2 level Concentration to initiate closure pending flesh testing results (cells per litre)
Alexandrium minutum	PSP	100	1,000
Alexandrium catenella	PSP	100	1,000
Alexandrium (unidentified)	PSP (possible)	100	1,000
Pseudo-nitzschia spp. (>50% total phytoplankton)	ASP (possible)	50,000	200,000
Pseudo-nitzschia spp. (<50% total phytoplankton)	ASP (possible)	100,000	500,000
Gymnodinium breve	NSP	1,000	5,000
Dinophysis acuminata	DSP (PTX2)	750	2,000
Dinophysis caudata	DSP (PTX2)	500	1,000
Prorocentrum lima	DSP (OA)	500	1,000

Biotoxin Monitoring

Shellfish samples for biotoxin analyses are only collected when phytoplankton monitoring indicates that a toxic algal species is increasing in abundance, or if the 'Category 1' level has been reached for a particular species. Usually only commercial shellfish species in the growing area are analysed. Sentinel bivalve species such as blue mussels or razorfish, which accumulate toxin more rapidly and to higher concentration in their flesh, are also tested on some occasions.

Closure and Re-opening Criteria

Closure Criteria

Harvesting is immediately suspended (or within 24 hours) at a shellfish growing area when one of the following criteria are satisfied:

- A toxic algal species is detected at the growing area with a cell concentration above a prescribed abundance (see Table 10, category 2 'Action Levels' above).
- Marine biotoxins (PSP, ASP, DSP or NSP) are detected in shellfish exceeding prescribed regulatory concentrations (Table 11). [These regulatory limits, with minor changes, are the same as those specified in the Australian New Zealand 'Food Standard Code'.]
- Cases of human illness, fitting the case definitions for PSP, NSP, DSP or ASP, have resulted from the consumption of shellfish from a particular growing area.

State Fisheries and Health Regulations control the culturing (Fisheries), harvesting (Health) and relaying operations (Fisheries) under the Fisheries Act 1982 (PIRSA) and the *Food Act* 1985 (South Australian Department of Human Services) (SADHS). These regulations allow the State to prohibit or restrict shellfish harvesting from any designated shellfish harvesting area in a public health emergency and to initiate the recall of product when necessary.

Table 11. SASQAP Marine biotoxin regulatory limits applicable to closing and re-opening criteria

Biotoxin type	Regulatory limit	Method of analysis
Paralytic Shellfish Poison (PSP)	≥ 80 µg saxitoxin equivalent/100 g of edible shellfish flesh	Mouse bioassay (1 hour max. observation time)
Neurotoxic Shellfish Poison (NSP)	≥ 20 mouse units (MU)/100 g edible shellfish flesh	Mouse bioassay (ether extraction & 6 hour max. observation time)
Amnesic Shellfish Poison (ASP)	≥ 20 µg/g edible shellfish flesh	HPLC
Diarrhetic Shellfish Poison (DSP) (except pectenotoxins)	≥ 20 µg (= 5 MU)/100 g edible shellfish flesh/ 100 g	Mouse bioassay (24 hour) or HPLC electrospray Mass Spectrometer
DSP (pectenotoxins)*	≥ 10 µg/100 g edible shellfish flesh	HPLC electrospray Mass Spectrometer

*An additional regulatory limit of 10 µg/100 g was prescribed for PTX2 and PTX2sa combined, as a precautionary measure, due to the production of these toxins by some *Dinophysis* spp. and their detection in South Australian shellfish. The regulatory limit was based on limited Australian toxin and mouse bioassay data as the human toxicity of pectenotoxins is currently unknown. Pipis contaminated with these same toxins were suspected to be responsible for two outbreaks of human illness in late 1997/ early 1998 in NSW. The 10 µg/100 g standard is now no longer used as the closure criteria, but an alternative standard is currently being assessed based on more recent Australian and New Zealand information.

Re-opening Criteria

The re-opening of a previously closed shellfish growing area can only occur after three main re-opening criteria are satisfied:

- The concentration of the relevant toxic algal species is decreasing and is lower than the critical prescribed abundance (Table 10,'Category 2 Level') in two consecutive samples collected at least six days apart.
- Biotoxin concentrations in the edible portion of shellfish are lower than prescribed regulatory limits (Table 11) in either two or three consecutive samples dependent on toxin type-
  PSP & ASP: 3 consecutive samples collected over a minimum period of 14 days.
  DSP & NSP: 2 consecutive samples collected over a minimum period of 7 days.
- No cases of human illness, fitting the case definitions for PSP, NSP, DSP or ASP, have been reported since the date of the first 'clear' biotoxin sample.

Program Administration

A Memorandum of Understanding, developed between Primary Industries and Resources South Australia (PIRSA) and the South Australian Department of Human Services (SADHS), documents the responsibilities of the two main organisations involved in marine biotoxin management.

The SASQAP Manager is responsible for the routine phytoplankton monitoring, biotoxin testing, and the investigation and evaluation of all toxic bloom events. He is also responsible for the closure and re-opening of shellfish growing areas, and for the early warning and official reporting of toxic bloom events to relevant statutory authorities / government agencies and the shellfish industry.

The Compliance Unit of Fisheries SA is responsible for patrolling harvesting areas during an area closure to ensure that no commercial or recreational product is harvested from the closed area, and to further warn the public by means of appropriate signage in the relevant area.

A network of liaison officers, elected by the growers, has been established in each shellfish growing area to provide a communication link between industry and government.

The Environmental Health Branch of SADHS, in consultation with the SASQAP Manager, is responsible for all media releases warning the public of harvesting area closures where there is a perceived risk from recreational harvesting of shellfish. SADHS is also responsible for the investigation of all suspected cases of shellfish poisoning and associated remedial actions.

Protocols for product recall have been developed which involve the cooperation and participation of SADHS, PIRSA, shellfish growers, processors, distributors and retailers of affected product. The SADHS is legally empowered to recall and detain contaminated product under the Food Act, 1985.

Internal Review
Annual reviews of the plan are undertaken.

5.5.1 Key Strengths of SASQAP Biotoxin Management Plan

(i) The current plan is both comprehensive and detailed with consideration given to most key requirements.
(ii) Additional individual management plans have been prepared for each shellfish growing area.
(iii) Surveys of dinoflagellate cysts in sediment have been undertaken at some shellfish growing areas to assess the potential biotoxin risk before phytoplankton monitoring commenced.
(iv) A full-time Phytoplankton/Biotoxin officer is employed as part of the program.
(v) The monitoring strategy is based on routine phytoplankton monitoring combined with some biotoxin testing.
(vi) All growing areas are monitored for all phytoplankton species.
(vii) 'Action levels' are used for the main toxic algal species to initiate biotoxin testing and to initiate a closure pending the results of toxin testing.
(viii) Comprehensive closure and re-opening criteria are in place to control harvesting.
(ix) Well-developed administrative procedures are documented, including official notification, communication, and media arrangements.
(x) A system of industry liaison officers has been established to provide a link between industry and government.
(xi) Results are entered into a central database allowing reporting as required.

5.5.2 Key Weaknesses in SASQAP Biotoxin Management Plan

(i) *Insufficient annual funding is available to the SASQAP Manager to increase frequency of phytoplankton monitoring and conduct necessary biotoxin analyses.*
Operational funding for 2000/01 of only $47,000 (plus 6 months initial funding of a staff member to conduct algal identifications and counts) is not sufficient to conduct all necessary phytoplankton and biotoxin monitoring. After paying independent sample collectors, freight, training, computing, etc. little funding remains available for analytical expenses. The funding allocation for 2001/02 is presently unclear.
(ii) *Insufficient contingency funding is available to the Manager of SASQAP to investigate toxic bloom events as they occur.*
The South Australian Department of Human Services (SADHS) has the responsibility for investigating all suspected cases of shellfish poisoning, and will pay all costs associated with these investigations. However, the Manager of the SASQAP does not have sufficient contingency funding available to enable him to fully implement the emergency sampling

protocol to prevent the harvesting of contaminated shellfish during the development of all toxic algal blooms. Additional funding is needed to increase the frequency and spatial coverage of phytoplankton monitoring and to conduct necessary biotoxin testing to define the severity of a toxic bloom event. As action must often be taken at very short notice, contingency funding should be made available at the start of each financial year. The Program Manager can apply for limited additional funds from a "Disaster Fund" within PIRSA, but only an extra $4,000 was received to investigate the lengthy *Dinophysis* blooms that appeared in South Australia in the year 2000.

(iii) *Inadequate public health protection from potential biotoxin contamination of commercially or recreationally harvested wildstock shellfish resources is in place.*

The primary focus of the plan concerns cultured shellfish grown in aquaculture zones and wild harvest shellfish are only included "in so far as they sometimes prove useful as sentinel species'' in areas where Pacific oysters are commercially grown. Wild harvest shellfish (scallops, pipis and cockles) accumulate algal biotoxins similarly to farmed shellfish and should therefore be included in the Biotoxin Management Plan. For example, there is a risk that blood cockles harvested commercially (and recreationally) from just outside the Port River may become contaminated with PSP toxins as potentially toxic *Alexandrium* blooms occur regularly in the river.

(iv) *Surveys for cysts of potentially toxic dinoflagellates in sediments within shellfish growing areas are incomplete.*

A survey for cysts of potentially toxic dinoflagellates in sediment was undertaken at six of the 11 shellfish growing areas in 1994, four years before the start of biotoxin monitoring in late 1998. All 11 growing areas should now be examined to complete the cyst survey and to satisfy the stated aim of conducting cyst surveys in sediment at each harvesting area at least once every five years. Cyst surveys are certainly of most relevance when conducted prior to or in the earlier stages of a monitoring program, as the monitoring strategy at each shellfish growing area can then be based on some form of initial risk assessment. Periodic surveys can then provide an insight into changing environmental conditions over time.

(v) *The documented phytoplankton sampling frequency, based on a limited knowledge of phytoplankton dynamics in each shellfish growing area, is inadequate to detect the presence and abundance of all potentially toxic species in a timely manner.*

It is argued in the April 2000 plan that phytoplankton samples be collected at least once per fortnight during "summer" (October to April) and at least monthly during "winter" (May to September). The sampling frequency may be increased if any toxic species are observed. However, the suggested sampling frequency is based on the results of only 18 months sampling including results for only one winter. Furthermore, there appears little justification at this stage to suggest that there is little risk of potentially toxic blooms occurring in winter. Several years' worth of data is required to draw such a conclusion due to possible large inter-annual variation. Population growth can be extremely rapid at any time of the year given favourable environmental conditions, and hence weekly sampling throughout the year is more appropriate. Sampling two or three times per week may be required for some toxic species, such as certain *Dinophysis* species, that can quickly reappear in the plankton and can cause shellfish to become unsafe when present in very low cell concentrations.

(vi) *Shellfish samples for biotoxin testing are only collected when phytoplankton monitoring indicates potential contamination of shellfish.*

Shellfish need to be tested for biotoxins on a routine basis in tandem with phytoplankton monitoring, not only as indicated by the phytoplankton results.

(vii) *Methods for phytoplankton sample collection are not provided in the Biotoxin Management Plan.*

Detailed methods are needed to ensure consistency in sampling methods.

(viii) *Phytoplankton 'Action Levels' should be set so they are appropriate for the area.*
Levels for other localities may be adopted in the first instance, in the absence of sufficient local data, but may be too restrictive. Although public health protection must always be the main priority, increased monitoring costs and unnecessary harvesting restrictions resulting from inappropriate action levels could adversely affect the shellfish industry. All 'action levels' should be re-examined annually to determine their appropriateness based on experience gained in local waters. Action levels for *Alexandrium* species and *Gymnodinium catenatum* should be set at the detection level of the phytoplankton enumeration method used to ensure the presence of these species triggers testing of shellfish for PSP toxins. In this instance a 'Category 2 level' of 2,000 cells/L for *Dinophysis acuminata* may be too high.

(ix) *Ambiguity exists regarding the application of the three closure criteria*.
The specified closure criteria are appropriate, but a shellfish growing area should be closed immediately (*not* within 24 hours) based on *any one of* the criteria. In addition, the regulatory limits given for ASP and DSP toxins should be expressed in terms of domoic acid and okadaic acid equivalents respectively (see ANZFA 'Food Standards Code'). (NB. This criterion has since been changed to the area being closed immediately.)

(x) *The key re-opening criteria are not readily apparent due to the mixing of criteria together with guidelines on their application.*
The three key re-opening criteria, based on toxic algal and biotoxin concentrations and the absence of human illness, should be separated from guidelines and procedural considerations as for the closure criteria. Three consecutive shellfish samples with biotoxin concentrations below regulatory levels, taken over a minimum period of 14 days, are specified for cases of PSP and ASP toxin contamination. For DSP and NSP toxins two consecutive samples taken over a minimum period of seven days are acceptable. A reason for the different requirement regarding the number of 'clear' consecutive samples for PSP and ASP toxins vis-à-vis DSP and NSP toxins, presumably because only the former toxins are potentially lethal, should be provided. The re-opening criteria should make provision for the identification of all toxins, so closures for other toxins do not need to be instigated immediately.

(xi) *Annual reviews of the Biotoxin Management Plan are required.*
Although the need for a review of the April 2000 plan by May 2001 was documented, reviews should be conducted annually to satisfy ASQAP requirements. Annual reviews are needed to re-assess the phytoplankton and biotoxin monitoring strategy, given a further twelve months algal and toxin data, and to evaluate the efficacy of management procedures and inter-agency communications during the most recent toxic bloom events.

(xii) *Important changes made to the Plan's operating procedures and closing/ re-opening criteria not documented.*
The Plan cannot be properly evaluated or audited, and its value is greatly diminished, if it does not reflect current practices. Changes made to the 'action levels' for toxic *Dinophysis* species and to the closing and re-opening criteria for pectenotoxins is one example. Any important variations made to standard operating procedures should be documented as an addendum to the Biotoxin Management Plan and formally inserted into the Plan at the next annual review.

5.6 Tasmanian Shellfish Quality Assurance Program (TSQAP)

Date of Latest Plan
May 2000 (currently under review).

Responsible Agency
Public & Environmental Health Service, Department of Health & Human Services (DHHS). Current Program Manager: Mr. Ray Brown.

Brief History of Biotoxin Surveillance
The 'Tasmanian Shellfish Quality Assurance Program' (TSQAP), the first of its type in Australia, was established in the early 1980's to enable the export of Tasmanian shellfish. The Division of Sea Fisheries (DSF) adopted the requirements and guidelines of the United States 'National Shellfish Sanitation Program' as an appropriate model. Responsibility for the operation of all aspects of the TSQAP, including biotoxin surveillance, was transferred from the Division of Sea Fisheries to the Public & Environmental Health Service of DHHS in 1991.

Biotoxin monitoring was initiated in 1986 following the development of an extensive bloom of the PSP-producing dinoflagellate *Gymnodinium catenatum* in the waters of south-eastern Tasmania. Fifteen shellfish farms in the area were closed for periods of up to six months. *Gymnodinium catenatum*, first discovered in southern waters in 1980, is believed to have been introduced into Tasmanian waters via ship's ballast water (Hallegraeff 1992).

Initially, the main focus of the biotoxin monitoring program involved the analysis of shellfish meat to determine the concentration of PSP toxins, and little phytoplankton monitoring was conducted. Shellfish were tested from farms from all around Tasmania, but particularly those from the danger area in the south east. In 1993 an 'Algal Watch' program was introduced to provide an earlier warning system of potentially harmful *G. catenatum* blooms. The current emphasis is placed on routine phytoplankton monitoring supported by relevant toxin testing of shellfish meat when necessary.

Fifteen years of monitoring results show that the PSP problem in Tasmania remains confined to the south east of the State; blooms of *G. catenatum* have only occurred in south-eastern waters. Supporting evidence is provided by CSIRO, who conducted sediment analyses from some shellfish growing areas around Tasmania to determine the geographic distribution of *G. catenatum* cysts (Bolch & Hallegraeff 1990). Viable cysts were found only in the Huon and Derwent Estuaries, the D'Entrecasteaux Channel and at Port Arthur.

Public health alerts were issued by DHHS each year a toxic bloom occurred. Two mild cases of paralytic shellfish poisoning (PSP) were reported following the collection and consumption of contaminated wild mussels during a massive bloom in 1993; a public health warning was in place at the time.

Biotoxin surveillance in Tasmania is mainly conducted to ensure the safety of all commercially cultured oysters and mussels. No monitoring is conducted to specifically protect the public harvesting wildstock shellfish in coastal waters, but health alerts are issued for broad areas based on monitoring data obtained for commercial shellfish growing areas. Commercial wild harvest shellfish such as scallops, clams, pipis and oysters are not routinely tested for biotoxins, although microbiologically clean areas where wild harvest shellfish (clams and oysters) are relayed for natural depuration are subject to phytoplankton monitoring.

The TSQAP is funded by the Department of Primary Industries Water & Environment (DPIWE) which provides funding for all direct costs (~$175000 p.a.) including that for biotoxin surveillance. Industry provides about 70% of total budget via a license fee component of $960 per marine farm or wild harvest license specifically for TSQAP. The budget for biotoxin monitoring/ management in FY 2000/2001 is $30,000 (salaries $20,000 and operating $10,000) excluding agency on-costs. No funding is contributed by some wild harvest shellfish industries (e.g. Bass Strait scallop industry) or the recreational sector.

Bivalve Shellfish Resources

- Pacific oyster (*Crassostrea gigas*)
- Native flat oyster (*Ostrea angasi*)
- Blue mussel (*Mytilus edulis*)
- Clams (*Katylesia* spp. and *Venerupis* spp.)
- Pipi (*Plebidonax deltoides*)
- Queen scallop (*Equichlamys bifrons*)
- Doughboy scallop (*Chlamys asperrimus*)
- Southern scallop (*Pecten fumatus*)

The above shellfish are widely distributed on east/south east and north/north west coasts of Tasmania. Recreational harvesting of oysters, mussels and clams is permitted in all areas except in the marine parks located at Tinderbox, Bicheno and Moulting Lagoon. There is also an ongoing health warning advising the public not to consume shellfish from the Tamar River estuary because of potential faecal contamination. The Marine Resources Division of DPIWE controls the recreational harvesting of scallops; open seasons are declared for specific areas subject to resource availability.

Commercial wild harvesting occurs in the following areas-

- Commercial scallops - Bass Strait.
- Clams/pipis - Recherche Bay, Hastings Bay, Esperance, Little Swanport, Georges Bay, and Ansons Bay.
- Native flat oysters - Georges Bay.

Designated Shellfish Growing Areas

Tasmanian shellfish growing areas including number of leases per area and maximum PSP concentration (μg/100 g of shellfish meat) recorded in area are as follows:

Growing Area	Number of Leases	Max. PSP (ug/100 g shellfish)	Shellfish grown
Montagu	5		
Big Bay	8	<50	oysters
Duck Bay	3	<50	oysters
Port Sorell	2	<50	oysters
Moulting Bay	9	<50	mussels
Great Oyster Bay	4		
Great Swanport	4	<50	oysters
Little Swanport	3	<50	oysters
Triabunna	3	<50	scallops
Mercury Passage	1	<50	scallops
Blackman Bay	7	<50	mussels
Dunalley Bay	3	<50	mussels
Norfolk Bay	5		

Growing Area	Number of Leases	Max. PSP (ug/100 g shellfish)	Shellfish grown
Eaglehawk Bay	1	<50	oysters
Garfish Bay/Dart Is.	5	<50	mussels
Port Arthur	1	<50	mussels
Little Norfolk Bay	1	<50	mussels
Pittwater	6	<50	mussels
Pipeclay Lagoon	9	58.5	oysters
Birchs Bay	1	331.7	mussels
Fleurty's Point	1	54.2	oysters
Long Bay Reef	1	<50	mussels
Great Bay	11	993.8	mussels
Simpson's Bay	3	467.0	oysters
Little Taylors Bay	3	<50	oysters
Cloudy Bay	1	50.9	oysters
Port Cygnet (Deep Bay)	2	18,429.9	mussels
Port Esperance	5	3,808.1	mussels
Hastings Bay	3	400.8	oysters
Recherche Bay	1	<50	oysters

Mussels from North West Bay (situated off the D'Entrecasteaux Channel) had a maximum PSP toxin concentration of 3,815 µg/100 g of mussel meat, although no shellfish are presently grown at this locality.

Phytoplankton and Biotoxin Monitoring

Phytoplankton Monitoring

The Huon River estuary is the area most affected by toxic blooms of *G. catenatum* in the State and all toxic blooms have first developed in this estuary before spreading to part of the D'Entrecasteaux Channel, Port Esperance, and North West Bay. Routine phytoplankton monitoring, conducted for the 'Algal Watch' program since 1993, has therefore been most intense in the Huon River estuary and adjacent waters.

All shellfish growing areas have been categorised as 'high', 'medium' or 'low' risk areas, based on the potential for toxic blooms of *G. catenatum* to occur in each area.

'High' risk areas:

- Huon River estuary (4 monitoring sites)
- Port Esperance (1 monitoring site)
- North West Bay (1 monitoring site)

'Medium' risk areas:

- Port Arthur
- Birches Bay
- Fleurtys Point
- Long Bay Reef
- Hastings Bay

Phytoplankton monitoring is conducted at least weekly at the six high-risk sites and the medium risk site at Port Arthur. The phytoplankton samples are collected by finfish farming companies (Tassal, Aquatas, and Huon Aquaculture Company), which conduct routine algal monitoring as part of their marine farm management operations. Plankton nets with a mesh aperture of 20 micron are used to collect the samples (sampling depth not stated). Sample analyses are conducted on fresh or

preserved samples by trained company staff who record the presence and relative abundance of potentially toxic species (particularly *G. catenatum*) and other algal species relevant to fish farming. *G. catenatum* is a large, rather conspicuous chain-forming species and therefore easily recognised. All results are routinely conveyed to the TSQAP Manager. Staff of the Woodbride Study Centre also conduct weekly sampling for much of the year at a site just north of Birches Bay. In addition, the CSIRO and University of Tasmania carry out occasional monitoring in the Derwent and Huon River estuaries; the TSQAP Manager is immediately notified if "significant" concentrations of *G. catenatum* are observed.

The medium risk areas (apart from Port Arthur) are sampled at least monthly, although the sampling frequency is increased to fortnightly or weekly sampling when the development of a toxic bloom is observed in the Huon. These samples, collected by TSQAP officers, are analysed by Analytical Services Tasmania. Low risk shellfish growing areas, i.e. those where no toxic blooms have been recorded and/or no cysts of toxic species have been found, are sampled only occasionally.

Biotoxin Monitoring
Shellfish are collected from shellfish growing areas by TSQAP officers whenever *G. catenatum* is observed and the TSQAP Manager is notified as per routine 'Algal Watch' reporting. Biotoxin monitoring results since 1986 have shown that toxic blooms of *G. catenatum* are most likely to occur after rainfall if the water temperature is above about 12°C. The danger period may therefore occur from spring to autumn. Only mussels are tested from shellfish growing areas where both oysters and mussels are cultured, as mussels take up PSP toxins more rapidly than oysters and accumulate more toxin in their tissues.

No routine analyses are conducted for ASP, DSP or NSP toxins.

Closure and Re-opening Criteria
Closure Criteria
There is currently only a single closure criterion used to control harvesting:

- Shellfish growing areas are closed for harvesting when the concentration of PSP toxins exceeds 80 μg saxitoxin equivalent/100 g of shellfish meat.

Closure criteria are not provided for ASP, DSP or NSP toxins, presumably because the risk from these types of poisoning is considered to be low. There are also no criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

The Director of Public Health "may require any agency, public authority or person to monitor the quality of water under its management or control" [Public Health Act 1997, Section 130 (1) & (2)]. He may also make an order "restricting or preventing the taking, harvesting or public supply of fish or shellfish from the water or which have been in the water" if satisfied that the quality of water is, or is likely to become, a threat to public health [Public Health Act 1997, Section 130 (1) (d)].

Re-opening Criteria
The re-opening of a previously closed shellfish growing area may occur when the following single criterion is satisfied:

- The concentration of PSP toxins in shellfish is below the prescribed regulatory limit of 80 μg saxitoxin equivalent/100 g of shellfish meat in two successive samples taken at least one week apart.

After a shellfish growing area is closed for shellfish harvesting, TSQAP officers usually coordinate the collection and testing of a further two shellfish samples to determine if the PSP concentration is increasing or decreasing. After this limited additional testing, the shellfish industry may arrange their own testing at any time to show that conditions are again safe. However, the re-opening of a closed area can only occur based on the collection and testing of at least two "clear" shellfish samples by TSQAP officers.

Program Administration
The DHHS has a close working relationship with the DPIWE and both government departments have recently signed a joint Memorandum of Understanding with the Australian Quarantine and Inspection Service (AQIS) concerning the sanitary control (including marine biotoxin control) of fresh and frozen molluscan shellfish intended for export from Tasmania. This document sets out the responsibilities of the two departments.

A TSQAP Management Committee currently oversees the operation of all aspects of the TSQAP including biotoxin management. Members of the Committee include the Director of Public Health (Chair), the TSQAP Manager, and representatives from DPIWE (2), Local Government Association (1) and the shellfish industry (4). Meetings are held about every two months.

The TSQAP Manager is responsible for the design and coordination of the 'Algal Watch' program, shellfish sampling and biotoxin testing, and the investigation of all toxic algal blooms in the marine environment. He is also responsible for the closure and re-opening of all shellfish growing areas, and for immediately notifying all affected shellfish farmers, shellfish processors and relevant government agencies (by phone and facsimile or certified mail). The Marine Farming Branch of DPIWE maintains a list of licensed 'marine shellfish farmers', while a list of 'shellfish processors' is maintained by TSQAP officers. The Manager also implements recall procedures when appropriate.

Marine Farm Inspectors of DPIWE and local Environmental Health Officers undertake surveillance and enforcement duties at relevant shellfish growing areas during a closure. Both DPIWE officers and the Tasmanian Police Force have powers under the Fisheries Act to control the relaying of wild harvest shellfish.

The Director of Public Health, acting on advice from the TSQAP Manager, is responsible for all media releases warning the public of the risk associated with recreational shellfish harvesting in contaminated areas during toxic algal blooms.

Internal Review
The plan is "constantly being modified and updated", although there is no specific date or frequency given for a complete program review. A major review of the Plan is currently in progress.

5.6.1 Key Strengths of TSQAP Biotoxin Management Plan

(i) The TSQAP Manager is very experienced in the area of marine biotoxin management having successfully controlled shellfish harvesting during blooms of *Gymnodinium catenatum* in the south east of the State over many years.
(ii) Considerable published research information is available on the distribution, population dynamics and toxicity of *Gymnodinium catenatum* in Tasmanian waters (see Hallegraeff, Bolch, Blackburn and colleagues).
(iii) Highly experienced research scientists working on toxic marine algae and marine toxins are available locally at the University of Tasmania and CSIRO.

(iv) Surveys of dinoflagellate cysts in sediment have been conducted in some shellfish growing areas around the State.

(v) An 'Algal Watch' program involving weekly phytoplankton monitoring by finfish farmers in the high-risk areas in the south east of the State is in place.

(vi) Strong legislative powers to enforce adequate marine biotoxin monitoring and to restrict or prevent the harvesting of shellfish if the quality of water is, or is likely to become, a threat to public health are in place.

5.6.2 Key Weaknesses in TSQAP Biotoxin Management Plan

(i) *Insufficient annual funding is available to the TSQAP Manager to run a satisfactory biotoxin management program.*

The 2000/01 budget of only $30,000 ($20,000 salary and $10,000 operating expenses) for biotoxin management is grossly inadequate given the potential marine biotoxin risk in Tasmania. It is commendable that the TSQAP Manager has been able to operate a creditable biotoxin monitoring program in the high-risk south-east area of the State. However, it is now necessary to expand the routine 'Algal Watch' program to other areas and to conduct marine biotoxin monitoring for toxins other than PSP toxins. The finfish industry in south-eastern Tasmania has routinely provided algal monitoring data at no cost to the TSQAP program, but the available data only provides coverage for a limited geographic area and limited algae species. Routine phytoplankton and biotoxin monitoring is relatively expensive but necessary to avoid possible food poisoning outbreaks and to protect the viability of Tasmania's commercial shellfish industries. The funding allocation for 2001/02 is currently unknown.

(ii) *No contingency funding is available to the TSQAP Manager to investigate toxic bloom events as they occur.*

There is no contingency funding allocation in the TSQAP budget to enable the Manager to conduct urgent unplanned phytoplankton monitoring and biotoxin testing during the development of a toxic algal bloom. Additional funding is needed to increase the frequency and spatial coverage of phytoplankton monitoring and to conduct extra biotoxin tests to define the severity and size of the bloom and to prevent the harvesting of contaminated shellfish. To be able to investigate any potential threat in a timely manner appropriate funding should be available prior to the event.

(iii) *Inadequate public health protection from potential biotoxin contamination of commercially or recreationally harvested wildstock shellfish resources is in place.*

Biotoxin surveillance conducted by the TSQAP primarily concerns the safety of commercially cultured oysters and mussels grown in designated shellfish growing areas and some re-seeded scallops grown in the Triabunna/Mercury Passage area. Monitoring results obtained for these areas can also be used to warn recreational harvesters when conditions are unsafe, but surveillance is not conducted in all recreational areas. No routine phytoplankton monitoring occurs in wild harvest shellfish areas, and no routine biotoxin testing is conducted on commercial wild harvest shellfish such as scallops, clams, pipis and wildstock oysters and mussels. However, domoic acid has been found in commercial scallops (mainly the viscera) harvested from Victorian and Commonwealth waters in Bass Strait and landed in Victoria. Scallops similarly harvested in Bass Strait and landed in Tasmanian ports should therefore be routinely tested for domoic acid and other toxins, especially if scallops are sold in the shell to Asian restaurants and overseas markets where whole tissues are consumed. All commercial wild harvest shellfish should be included in the biotoxin-testing program to provide necessary public health protection.

(iv) *Key elements and considerable detail are lacking in the current Plan*.
Although an extensive review of the April 2000 Plan is in progress, the present evaluation can only be made on the currently available document that has essentially formed the basis of routine operations for some years. One key missing element is a complete list of all designated shellfish growing areas in the State, including the names and/or site numbers of smaller sub-areas within each major growing area. Exact locations of all sampling sites are also required. Relevant maps would be helpful, particularly in situations where one sampling site is chosen to represent several specific areas or leases within a large shellfish growing area. Consideration should be given to all components outlined in the "suggested contingency plan for the control of marine biotoxins" contained in the 'Operations Manual of the Australian Shellfish Sanitation Control Program'. Greater detail is needed throughout the plan, including detailed procedures and guidelines, so that appropriate biotoxin management could continue satisfactorily in the absence of the TSQAP Manager.

(v) *Insufficient biotoxin surveillance is conducted in shellfish growing areas situated outside the high-risk south-eastern corner of the State.*
Most of the phytoplankton monitoring and toxin testing conducted to date has by necessity been restricted to the south-eastern region, largely because of the threat posed by regular blooms of *Gymnodinium catenatum* and the resultant contamination of cultured oysters and mussels with PSP toxins. However, other potentially toxic species are more widely distributed around the State and hence phytoplankton monitoring and relevant biotoxin testing should be conducted in all shellfish growing areas. Note: an expansion of the 'Algal Watch' program is already planned.

(vi) *Insufficient attention is given to potentially toxic species other than* Gymnodinium catenatum, *which has implications concerning sampling sites and the frequency of monitoring*.
A thorough investigation is required on the distribution, abundance and toxicity of other potentially toxic species such as *Pseudo-nitzschia* species, *Dinophysis* species, *Prorocentrum lima, Karenia mikimotoi (=Gymnodinium mikimotoi)* types, *Karenia* cf *brevis (=Gymnodinium* cf *breve*) and *Alexandrium* species. The current sampling sites around the State were largely selected to provide an early warning of toxic *G. catenatum* blooms. However, the number and location of phytoplankton sampling sites, and the frequency of sampling, will need to be expanded to conduct the above investigation.

(vii) *Absence of biotoxin testing of shellfish for toxins responsible for ASP, DSP and NSP*.
Essentially all biotoxin testing to date has involved mouse bioassays for PSP toxins. Biotoxin analyses for ASP, DSP and NSP should also be conducted at appropriate times.

(viii) *Quantitative phytoplankton data are not provided in the current 'Algal Watch' program.*
Routine phytoplankton monitoring to date has involved the collection and analysis of net-tow samples to provide presence/absence or relative abundance information on potentially toxic genera or species. There needs to be more emphasis placed on quantitative rather than qualitative sampling techniques. Much of the data has been obtained from the finfish industry, which conducts routine phytoplankton analyses as part of their normal business operations, but does not include data for all potentially toxic species. The collection of quantitative water samples and the provision of cell concentration estimates for a complete list of potentially toxic species, as proposed in a new expanded 'Algal Watch' program, should overcome this current deficiency. External service providers will conduct the water sample analyses. 'Action levels' for phytoplankton should then be provided for all potentially toxic species. Reviews should be conducted annually in the future to ensure that the plan reflects current operating practice at all times.

(ix) *Additional closure criteria, in addition to that for PSP toxins, are necessary to ensure adequate public health protection.*

There is only a single 'closure criterion' in the current plan: "Marine farms will be closed for harvesting when the level of biotoxin [PSP toxins] in the shellfish meat exceeds the equivalent of 80 µg saxitoxin/100 g of meat". This is inadequate given the somewhat limited investigation of toxic algal species potentially able to cause ASP, DSP and NSP in Tasmanian waters. Criteria should be provided for domoic acid (ASP), brevetoxins (NSP) and DSP toxins. Standards for all four main toxin types are now included in the Australian New Zealand 'Food Standards Code'. A closure should also occur when the cell concentration of a toxic algal species exceeds action level 2, the level prescribed to initiate a closure pending the results of toxin testing of shellfish meat. Further closure criteria should be added based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

(x) *The present single re-opening criterion requires clarification and amendment.*
Although implied, the present single 're-opening criterion' does not specify the toxin type, *i.e*. PSP toxins expressed in terms of saxitoxin equivalent. Furthermore, it is commonly accepted practice, at least in the case of potentially lethal toxins, to conduct biotoxin tests on three consecutive samples of the same shellfish species collected over a minimum period of 14 days. Two consecutive samples taken 7 days apart may not provide sufficient protection in shellfish growing areas where there is considerable spatial variation in toxin concentration and only one sampling site representing a broad area.

(xi) *Complementary re-opening criteria matching the additional closure criteria are required.*
Complementary re-opening criteria should be added to cover the situation where ASP, DSP or NSP toxins have previously caused a shellfish growing area to be closed. The concentration of the toxic algal species responsible for the closure should also be clearly decreasing and remain below the prescribed 'action level' for that species. Lastly, no cases of human illness, fitting the accepted case definitions for PSP, ASP, DSP or NSP, should have resulted from the consumption of any shellfish harvested from within or adjacent to the closed area since the date of the first 'clearance' sample.

5.7 Victorian Shellfish Quality Assurance Program (VSQAP)

Date of Latest Plan
No comprehensive marine biotoxin management plan is available for Victoria at the present time. The most relevant document remains the Marine Science Laboratories Internal Report No. 187, entitled "Surveillance of toxic marine algae and biotoxins in shellfish in Port Phillip Bay", published in 1990. This 'Plan' sets out the general approach taken by the Victorian Shellfish Quality Assurance Program (VSQAP) concerning biotoxin surveillance for many years. The current routine monitoring service provider for Fisheries Victoria is Water ECOscience (WES), who have recently developed several draft sampling and testing protocols that describe present operating procedures for the three main toxic genera.

Responsible Agency
Fisheries Victoria, Department of Natural Resources and Environment (DNRE), is the State Shellfish Control Agency.
Current Program Manager: Anthony Forster (Aquaculture Manager).
The Marine and Freshwater Resources Institute (MAFRI) has an advisory role as documented in annual service agreements with Fisheries Victoria; the relevant MAFRI officer is Neil Hickman.

Brief History of Biotoxin Surveillance
Victoria has the longest history of regular marine biotoxin monitoring and has recorded the four main biotoxin types (PSP, ASP, DSP and NSP) over time. A large number of biotoxin analyses (mainly for PSP toxins and domoic acid) have been conducted in the State since biotoxins were first detected in January 1988.

The VSQAP was first established in September 1987 to ensure the safety of commercially cultured blue mussels grown at four aquaculture zones (Clifton Springs/Point Richards, Grassy Point, Beaumaris and Dromana) in Port Phillip Bay and one zone at Flinders in neighbouring Western Port. Wild harvest mussels (from the Gippsland Lakes in eastern Victoria) and scallops from Port Phillip Bay and Bass Strait were included in the program at a later date. MAFRI (located at Queenscliff) have managed or have assisted in the management of the VSQAP for Fisheries Victoria since its inception to present day, and were responsible for all operations for the first eleven years of the program. WES took over the responsibility for sample collection and analysis and some reporting functions for the mussel culture industry only in March 1998, operating under tender since July 1999.

Surface phytoplankton and mussels for PSP analyses have been regularly collected in Port Phillip Bay and Western Port since 1987, with fortnightly sampling conducted from 1990. MAFRI consequently have an extensive phytoplankton database for Port Phillip Bay and have analysed data for the five-year period from March 1990 to February 1995 (Magro *et al.* 1996 and Arnott *et al.* 1997). Six species of *Alexandrium* have been observed in Port Phillip Bay – *A. catenella* and *A. tamarense* (both toxic), *A. minutum* and *A. ostenfeldii* (potentially toxic), and *A. pseudogonyaulax* and *A. margalefi* (non-toxic). During the long period of monitoring shellfish farms, PSP toxins were only found at low levels a period of a few weeks in one area associated with a winter bloom of *A. tamarense*.

In contrast to the offshore mussel farming areas, recreational gathering of wild stock mussels from Hobson's Bay does present a potential health risk with *Alexandrium catenella* blooms having occurred in this region and surrounding waters in summer or autumn. Hobsons Bay is a considerable distance north of Beaumaris, the most northerly aquaculture zone in Port Phillip Bay,

and the commercial wild harvest of mussels is not permitted in Hobson Bay. Major blooms of *A. catenella* in the summer of 1988, 1992, 1994, and 1995 led to public health alerts warning the public not to collect and eat shellfish from contaminated areas (Arnott 1998). A maximum PSP toxin concentration of 10,010 μg/100 g was recorded in wildstock mussels from Port Melbourne in January 1992. This concentration was 125 times greater than the food standard of 80 μg saxitoxin equivalent/100 g. Low concentrations of PSP toxins were detected at Beaumaris on several occasions but never above the regulatory food standard.

Aquaculture zones in Port Phillip Bay have only been closed on one occasion due to shellfish contamination by PSP toxins. The harvesting of cultured mussels at Clifton Springs/Port Richards and Grassy Point, off the Bellarine Peninsula, was temporarily suspended in July 1993 when PSP concentrations reached a maximum of 275 μg/100 g. The causative organism, *A. tamarense*, was observed only in low numbers. This species reappeared in the same area in the winter of 1994, but the maximum PSP concentration was only 64.5 μg/100 g. No PSP-toxins have been detected in mussels from Western Port.

Routine biotoxin surveillance has also been conducted at times for the small commercial wild harvest mussel industry in the Gippsland Lakes and for the 'roe-on' scallop industry operating in both Port Phillip Bay and Bass Strait (note scallop harvesting is now banned in the Bay). No PSP toxins have been detected.

In July 1992 large numbers of *Gymnodinium catenatum* cysts were observed in the water column and in sediment at Lorne in western Victoria. As cysts can be very toxic a public health warning was immediately issued. Follow-up sampling revealed that *G. catenatum* and/or *A. tamarense* were present in many ports or harbours sampled along both the eastern and western Victorian coastline. Both cysts and motile cells were observed. Relatively low PSP-toxin concentrations were also found in the 'gut' tissues of abalone (max. 123 μg/100 g) and rock lobsters (max. 180 μg/100 g), while concentrations below the food standard were found in abalone meat (max. 66 μg/100 g). Abalone and rock lobsters are not filter feeders so the exact route of toxin entry is not known.

A major bloom of the potentially toxic diatom *Pseudo-nitzschia pseudodelicatissima* was observed in Port Phillip Bay for four months in 1991/1992, but no domoic acid was recorded either then or during subsequent fortnightly toxin monitoring of cultured mussels and scallops from the Bay. All Victorian strains of *P. pseudodelicatissima* have been consistently non-toxic. However, the potentially toxic species *P. multiseries* and *P. australis* have been recorded in the Bay in recent years, and hence close attention must be given to all *Pseudo-nitzschia* blooms.

Domoic acid has, however, been detected in low concentrations in scallops from both Victorian and Commonwealth waters in Bass Strait (Arnott *et al.* 1994). The initial finding in June 1993 was the first record of domoic acid present in any Australian shellfish. Subsequent testing over five months found a high percentage of positive results with concentrations ranging from 0.2 to 1.2 μg/g in the edible 'roe-on' portion and 1.8 to 26.2 μg/g in the viscera; the Australian New Zealand regulatory limit is currently 20 μg/g. As a precautionary measure the then Victorian Department of Health and Community Services issued an order in July 1993 forbidding "the sale or supply of scallops other than scallops which had been opened and from which the viscera had been removed and discarded". Extremely low concentrations have been found during later monitoring but only in the viscera. Gilgan *et al.* (1990) observed that domoic acid in contaminated Atlantic scallops was not cleared in animals held for over four months, and noted that the toxin was strongly retained in the digestive gland. The source of the domoic acid in Bass Strait is unknown.

Neurotoxic shellfish poisoning (NSP) was reported in Victoria, the first and only record for Australia, in 1994 (Arnott 1998). An unknown number of people (including a general practitioner) became extremely ill after eating wildstock mussels harvested from the Tamboon Inlet on the Gippsland coast. Cases of illness had apparently also occurred during the previous summer. A cell concentration of 84,000 cells per litre of *Karenia* cf *brevis* (=*Gymnodinium* cf. *breve*) was found on 21 January 1994, and a corresponding mussel sample had a brevetoxin concentration of 27.5 MU/100 g of mussel meat. A public health warning was immediately issued and warning signs were erected at the Inlet. The algae soon decreased in concentration to 3,900 cells per litre by 9 February 1994 when no further toxin was detected. The Australian New Zealand Food Authority regulatory limit for brevetoxin is 20 MU/100 g of shellfish meat.

Several potentially toxic *Dinophysis* species are known to have been present in Port Phillip Bay for several decades now; however, until recently the Victorian strains have been considered to be non-toxic. No cases of DSP have ever been reported. In the year 2000 PTX2sa and small concentrations of PTX2 and okadaic acid (OA) were detected in Port Phillip Bay cultured mussels during a bloom of *D. acuminata* (Neil Hickman, MAFRI, Victoria, Australia, pers. comm.). The maximum PTX2sa concentration was 29.0 µg/100 g, which occurred at a time when the concentration of *D. acuminata* was 1,360 cells per litre. The Victorian mussel culture industry agreed to adopt a voluntary closure when cell concentrations were greater than 2,000 cells per litre. One closure, at the Dromana Bay aquaculture zone, subsequently occurred in September 2000; the combined pectenotoxin and okadaic acid toxin concentration in mussel meat was later shown to be less than 20 µg/100 g. The human toxicity of pectenotoxins is currently unknown. The cases of DSP that occurred in NSW following the consumption of pipis contaminated with the same pectenotoxins has not been satisfactorily confirmed. The Australian New Zealand Food Authority regulatory limit for DSP toxins is 20 µg okadaic acid equivalent/100 g.

Phytoplankton monitoring and biotoxin testing is still routinely conducted to ensure the safety of mussels cultured in Port Phillip Bay and Western Port. However, no monitoring has been undertaken in the high-risk area for PSP toxins in the north of the Bay, and at other areas where recreational shellfish harvesting may occur, since the then Department of Health and Community Services withdrew from the VSQAP some years ago. Furthermore, there is no current biotoxin monitoring of Bass Strait scallops or commercially or recreationally harvested wildstock mussels from the Gippsland Lakes.

In the past VSQAP has been funded 100% by Fisheries Victoria. However, industry will be expected to contribute 33% of required funding if a new Fisheries Regulation currently awaiting Parliamentary approval is passed. A levy is proposed for all holders of a new "Type A Bivalve Aquaculture Licence". From figures supplied Fisheries Victoria, it is estimated that about $75,000 is currently spent annually on biotoxin surveillance in Victoria; considerably higher amounts were spent in earlier years.

Bivalve Shellfish Resources

- Native flat oyster (*Ostrea angasi*)
- Blue mussel (*Mytilus edulis*)
- Commercial scallop (*Pecten fumatus*) [roe-on]
- Pipis (*Plebidonax deltoides*)
- Razorfish (*Pinna bicolor*)
- Various clams and cockles

The blue mussel is the only commercially cultured bivalve species presently grown in Victorian waters. In past years Pacific oysters have been cultured in salt ponds on a land-based system,

situated on the western coastline of Port Phillip Bay, where no seawater is returned to Bay. There is a current Government ban on the culture of Pacific oysters in Victorian coastal waters for environmental reasons. Pacific oysters have never been included in the VSQAP. Industry growth trials with scallops, conducted mainly in mussel growing areas under VSQAP control, are also well advanced.

There are proposals to harvest both scallops and wildstock native flat oysters and hold them in approved shellfish growing areas in Port Phillip Bay for several months prior to export. Other bivalve species are also being assessed as to their suitability for culture.

Scallops (roe-on) are harvested from Victorian and Commonwealth waters in Bass Strait, while blue mussels are harvested at the marine end of the Gippsland Lakes.

All edible bivalves are recreationally harvested by the public but some restrictions apply for shellfish protection reasons.

Designated Shellfish Growing Areas

Blue mussels are or have been cultured at the following sites:

Port Phillip Bay	Clifton Springs/ Port Richards
	Grassy Point (Portarlington)
	Dromana Bay
	Balcombe Bay [currently inactive]
	Beaumaris [currently used for spat collection only]
Western Port	Flinders Bight

Phytoplankton and Biotoxin Monitoring

Phytoplankton Monitoring

Phytoplankton net tow samples and quantitative water samples are collected fortnightly at each of the mussel growing areas in Port Phillip Bay and at Flinders in Western Port. A scan of the net sample is initially conducted to provide relative abundance data for the common genera and species and to detect the presence of any toxic species. If any toxic or potentially toxic species are observed, an estimate of the cell concentration for the relevant species is determined from the quantitative water sample. A list of the toxic and potentially toxic species is maintained and updated as necessary. Provision is made to increase the frequency of sampling whenever toxic species are detected.

Very detailed and comprehensive draft management protocols, prepared by WES for Fisheries Victoria, have been developed for *Pseudo-nitzschia* spp., *Alexandrium* spp., and *Dinophysis acuminata* and other potentially toxic *Dinophysis* species. Table 12 shows the trigger levels for phytoplankton species.

Biotoxin Monitoring

Mussel samples are collected every fortnight, for biotoxin analysis, from each of the mussel growing areas in Port Phillip Bay and at Flinders in Western Port. PSP analyses are conducted on samples from all sites, while domoic acid analyses are routinely conducted on samples collected from Clifton Springs and Flinders.

Table 12. VSQAP phytoplankton trigger levels

WATER ECOscience	Phytoplankton Abundance Triggers for the VSQAP (cells/L)			
Alga species	**Toxin**	**Warning Issued**	**Tissue Testing**	**Harvest Suspension Pending Toxin Analysis**
Pseudo-nitzschia spp.(<50% total phytoplankton)	ASP	100,000	300,000	500,000
Pseudo-nitzschia spp. (>50% total phytoplankton)	ASP	50,000	100,000	200,000
Rhizosolenia cf *chunii*	Bitter Taste	10,000	N/A	20,000 Level 2 Warning
Alexandrium catenella	PSP	100	Routine or 100	*500
Alexandrium minutum	PSP	100	Routine or 100	*500
Alexandrium tamarense	?PSP	100	Routine or 100	*500
Gymnodinium catenatum	PSP	Presence	Presence	*500
Alexandrium margalefi	haemolytic?	100	Routine or 100	500
Dinophysis acuminata	DSP	1,000	1,000	2,000
Dinophysis caudata	DSP	1,000	1,000	2,000
Dinophysis fortii	?DSP	1,000	1,000	2,000
Prorocentrum lima	?DSP	1,000	1,000	2,000
Karenia cf brevis (=Gymnodinium cf *breve)*	NSP	Presence	*1,000	*5,000
Gymnodinium/Karenia spp. (NOT *catenatum, mikimotoi* or *brevis*)	??NSP	5,000	5,000	? Not decided
Prorocentrum minimum	?	1,000	1,000	2,000

* New Zealand trigger adopted for now until more information is available for PPB

NOTE: Harvest suspension pending biotoxin analysis is merely precautionary; suspension / resumption of harvesting will be determined by toxin levels as noted below.

Closure and Re-opening Criteria

Closure Criteria

Mussel harvesting is immediately suspended at a shellfish growing area when one of the following criteria are satisfied:

- A toxic algal species is detected at the growing area with a cell concentration exceeding a specified abundance for tissue testing (Table 12),
- Marine biotoxins are detected in mussel meat exceeding the prescribed regulatory limits (Table 13).

Table 13. VSQAP Marine Biotoxin Regulatory limits

WATER ECOscience		Tissue Biotoxin Regulatory Limits for the VSQAP		
Toxin Class	**Units**	**Regulatory Limit**	**Method**	**Limit of Detection**
PSP	µg/100 g	80	Bioassay	26
ASP (domoic acid)	µg/g (ppm)	20	HPLC	0.5 – 1.0
DSP	µg/100 g	20	HPLC/MS	0.3
NSP	MU/100 g	20	Bioassay	10

MU = mouse units

An even lower concentration of only 16 µg DSP toxins/100 g is suggested to be a more appropriate standard in certain situations. Furthermore, a domoic acid concentration of 10 µg/g is suggested to be an appropriate trigger to suspend harvesting during toxic *Pseudo-nitzschia* blooms; this is only 50% of the regulatory standard.

There are no criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

Under Section 152 (1) (d) of the Fisheries Act 1995 the Minister may, by a fisheries notice in relation to any fishery, "close to harvesting for the period of time specified in the notice any shellfish beds or shellfish farms, where necessary in response to adverse environmental conditions".

Re-opening Criteria
The re-opening of a previously closed shellfish growing area can only occur when three successive mussel samples, taken over a 14-day period, satisfy the criteria shown in Table 14 following re-opening criteria:

Table 14. VSQAP Re-opening Criteria

Toxin	Harvest Resumption
ASP	<10 µg/g domoic acid for 3 successive samples over 14 days; phytoplankton abundance not rising.
PSP	<80 µg/100 g PSP for 3 successive samples over 14 days; phytoplankton abundance not rising.
DSP	<16 µg/100 g DSP for 3 successive samples over 14 days; phytoplankton abundance not rising.
NSP	< 20 MU/100 g for 3 successive samples over 14 days; phytoplankton abundance not rising.
Bitter Taste	Harvesting suspended/resumed by growers depending on taste of mussels.

Note that the DSP and ASP toxin concentrations are lower than the prescribed regulatory limits provided in the Australian New Zealand 'Food Standards Code'. Guidelines were provided concerning the application of the criteria.

Program Administration/Internal Reviews
There is no biotoxin management plan currently available for Victoria and hence details concerning program administration and the timing or frequency of internal reviews are not provided.

5.7.1 Key Strengths of VSQAP Biotoxin Surveillance

(i) Long history of routine phytoplankton monitoring conducted at mussel growing areas in Port Phillip Bay and Western Port.
(ii) Long history of routine biotoxin monitoring for PSP toxins and domoic acid in cultured mussels grown in Port Phillip Bay and Western Port.
(iii) Extensive phytoplankton and biotoxin (PSP toxins and domoic acid) databases available at MAFRI.
(iv) Considerable published research information available on toxic algal blooms and the distribution and abundance of toxic marine algae in Port Phillip Bay.
(v) Considerable phytoplankton and biotoxin data obtained during earlier monitoring conducted for the commercial wild stock mussel industry in the Gippsland Lakes.
(vi) Considerable biotoxin data (especially for PSP toxins and domoic acid) available for scallops harvested from both Port Phillip Bay and Bass Strait.
(vii) Limited data available on PSP toxins detected in rock lobster, abalone and other marine fauna present in Victorian coastal waters.
(viii) Surveys of toxic algal cysts conducted in Port Phillip Bay and Victorian coastal waters.

5.7.2 Key Weaknesses in VSQAP Biotoxin Surveillance

(i) *Insufficient annual funding is available to the VSQAP Manager to conduct routine phytoplankton monitoring at all aquaculture areas and to conduct necessary biotoxin analyses.*

Contingency funding should also be provided to investigate the development of all potentially toxic algal blooms. Research funding is also required to conduct strategic biotoxin analyses of algal and shellfish samples to determine which algal species produce which toxins, and to determine appropriate 'threshold levels' for all potentially toxic algal species in Victorian waters.

(ii) *There is no comprehensive biotoxin management plan available for Victoria at the present time.*

A comprehensive biotoxin management plan is urgently needed in Victoria to provide adequate public health protection for all consumers of Victorian marine bivalve shellfish. A State plan is also needed to satisfy the biotoxin control requirements of the Australian Shellfish Quality Assurance Program (ASQAP) so that exports of Victorian shellfish may occur. Attention should be given to all components outlined in the "Suggested contingency plan for control of marine biotoxins" contained in Appendix VI of the ASQAP 'Operations Manual'. Although not formally documented, it appears that adequate phytoplankton and biotoxin monitoring is being conducted at mussel culture areas. However, a contingency plan is required to guide essential management operations when a toxic bloom does occur. Due to the lack of a current plan, biotoxin management arrangements in Victoria could not be fully evaluated for the present review.

(iii) *Inadequate public health protection from potential biotoxin contamination of commercially harvested scallops from Bass Strait and wildstock mussels from the Gippsland Lakes is in place.*

Biotoxin surveillance conducted by the VSQAP primarily concerns the safety of commercially cultured mussels grown in designated shellfish growing areas in Port Phillip Bay and Western Port. No routine biotoxin monitoring is currently conducted for scallops harvested from Victorian and Commonwealth waters in Bass Strait and landed in Victoria ports. However, domoic acid has already been found in the roe and viscera of commercial scallops harvested from Bass Strait several years ago and hence the ongoing monitoring of scallops for domoic acid and other potential toxins is considered to be essential. Scallops sold in the shell to Asian restaurants and overseas markets, where whole tissues may be consumed, provide the highest risk. But 'roe-on' scallops may also contain potentially harmful toxin concentrations. Routine phytoplankton and biotoxin monitoring to protect consumers of commercially or recreationally harvested wildstock mussels from the marine end of the Gippsland Lakes also ceased some years ago. Although no biotoxins were found during previous monitoring, the Gippsland Lakes system is a potential high-risk environment. Toxic algal species (including PSP and brevetoxin producers) have been found in Victoria's eastern coastal waters, and domoic acid occurs in scallops harvested off the Gippsland Lakes. All commercial wild harvest shellfish should be included in the biotoxin testing program to provide necessary public health protection.

(iv) *No public health protection is provided for consumers of mussels harvested from extremely high-risk areas in northern Port Phillip Bay.*

Regular fortnightly biotoxin surveillance was conducted for many years in northern Port Phillip Bay in areas far removed from the closest aquaculture zone in the Bay. Routine monitoring ceased when the Department of Health and Community Services (now the Department of Human Services) withdrew from the VSQAP. However, northern Port Phillip Bay is an extremely high-risk area where toxic blooms of *Alexandrium catenella* have been observed and where a PSP toxin concentration 125 times greater than the

regulatory limit specified in the 'Food Standards Code' has been detected. An outbreak of PSP arising from the consumption of recreationally harvested shellfish from Port Phillip Bay would cause considerable damage to the image of all shellfish industries.

(v) *Annual reviews of the Biotoxin Management Plan are required.*
Annual reviews are needed to re-assess the phytoplankton and biotoxin monitoring strategy, given a further twelve months' algal and toxin data, and to evaluate the efficacy of management procedures and inter-agency communications during the most recent toxic bloom events. The Biotoxin Management Plan should reflect standard operating procedures at all time.

5.8 Western Australian Shellfish Quality Assurance Program (WASQAP)

Date of Latest Plan
Biotoxin management arrangements are included in the 'Operations Manual' of the WASQAP. A 1999 version was provided for evaluation. The Operations Manual is currently under review, and the revised 2001 Operations Manual is due to be issued at the end of June 2001.

Responsible Agency
The Health Department Western Australia (HDWA) and Fisheries Western Australia (FWA) are jointly responsible for the administration of the Western Australian Shellfish Quality Assurance Program (WASQAP), including biotoxin management of commercial aquaculture areas.
Current Program Manager: Kim Leighton (HDWA).

Brief History of Biotoxin Surveillance
Biotoxin monitoring is an important component of the WASQAP and has been conducted in commercial aquaculture areas of Western Australia since 1994. The program was established primarily to ensure the safety of cultured mussels and oysters grown at the main aquaculture lease sites located in Cockburn Sound and Oyster Harbour at Albany.

The initial approach involved both routine quantitative phytoplankton monitoring and the routine testing of shellfish for PSP toxins using a mouse bioassay. Since January 2000 more intensive bimonthly phytoplankton sampling has been conducted and abundance estimates of all phytoplankton taxa are provided with a considerably lower detection limit than previously occurred. Relevant biotoxin testing, either for PSP, ASP, DSP or NSP toxins, is conducted only when a potentially toxic algal species is present in an aquaculture area in concentrations above specific "threshold levels" specified for the particular toxic species (Table 13).

Although a number of potentially toxic algal species have been observed in Western Australian waters, no toxic algal blooms have occurred in the commercial aquaculture areas to date. Furthermore, no PSP toxins have been detected in shellfish, no growing area closures have been necessary and no cases of shellfish poisoning have been reported. However, DSP toxins were detected (by ELISA method) in cultured mussels from Oyster Harbour on twelve occasions in 2000, when *Dinophysis acuminata*, *Prorocentrum lima*, and *P. mexicanum* were present among the phytoplankton. All three species have been shown to produce DSP toxins in other Australian States and/or overseas. Okadaic acid, PTX2 and PTX2sa (the latter particularly abundant) were detected by LC-MS in one mussel sample collected when *D. acuminata* was quite abundant.

No phytoplankton monitoring is conducted specifically to protect recreational harvesters of marine shellfish, although monitoring data obtained by the WASQAP and by the Waters and Rivers Commission for rivers and estuaries provides some public health protection. Public health alerts concerning toxic marine algae have not been necessary so far.

Three commercial licenses have been issued for the wild harvest of blue mussels in Cockburn Sound. All three licenses are currently inactive but when operational the license holders must participate in the WASQAP. Wild harvest areas are not specifically targeted for routine phytoplankton or toxin monitoring; however, bimonthly monitoring is already being conducted at two localities in Cockburn Sound. 'Roe-on' saucer scallop and pearl oyster meat is not currently tested.

Biotoxin surveillance in Western Australia is funded jointly by industry (mussel and oyster growers) and government. Industry collects the appropriate samples and is responsible for sample transport and for the cost of routine phytoplankton sample analyses. The government (DHWA & FWA) is responsible for program administration including preparation of the 'Operations Manual' of the WASQAP, general data and program management, program review and report writing. The government also pays for necessary biotoxin testing in the event of a toxic algal bloom. Direct costs to industry in 2000/2001 are about $7,500-$10,000.

Bivalve Shellfish Resources

- Western rock oyster (*Saccostrea glomerata*)
- Native flat oyster (*Ostrea angasi*)
- Pearl oyster (*Pinctada maxima*)
- Blue mussel (*Mytilus edulis*)
- Ballots saucer scallop (*Amusium balloti*)
- Southern scallop (*Pecten fumatus*)
- Razorfish (*Pinna bicolor*)
- Pipi (*Plebidonax deltoides*)
- Giant clams (*Tridacna* spp. and *Hippopus hippopus*)

Commercial wild harvest fisheries are restricted to blue mussels (in Cockburn Sound) and saucer scallops. The main scallop-producing areas are Shark Bay (catch range of 100-4,000 t) and a region around the Abrolhos Islands (10-500 t), while smaller catches are taken from the 'South West Trawl Managed' fishery (5-50 t) and 'South Coast Inshore Trawl' fishery. Until 1978 all scallops were landed whole and processed ashore, but since then they have usually been processed at sea and landed as frozen meats. Small quantities of 'roe-on' scallops may be supplied to the gourmet seafood market.

Designated Shellfish Growing Areas

Growing Area		Number of Leases
Cockburn Sound	Kwinana Grain Terminal (KGT)	7
	Southern Flats (SF)	7
	Northern Garden Island	2
Albany	Oyster Harbour (OH)	3
	King George Sound (KGS)	1*
	Mistaken Island	6**

* Mussel culture leases currently inactive.

** Area not yet classified but mussel culture currently at one lease.

Phytoplankton and Biotoxin Monitoring

Phytoplankton Monitoring

During 1999, a 1 litre surface water sample and a phytoplankton net tow sample were collected monthly at one Kwinana Grain Terminal site (KGT3) in Cockburn Sound, and approximately monthly from May to December at one site in Oyster Harbour (OHA1) at Albany. Sampling details are given for Cockburn Sound where a phytoplankton net (mesh aperture 20 μm) was towed for five minutes each side of the lease area, and 1 or 2 vertical hauls were conducted from the bottom to the surface in the middle of the lease area. The net samples were pooled prior to examination to detect the presence of potentially toxic algal species. Phytoplankton cell counts were made by filtering 500 ml of the 1 litre water sample through a 0.45 μm millipore filter, resuspending the filtrate in 10 ml of seawater, then counting the cells in a sub-sample of known volume on a Neubauer haemocytometer. The detection limit using this technique was about 1,200-2,000 cells per litre.

The sample collection and analysis methodology changed in January 2000, when regular bimonthly sampling was initiated at the above two sampling sites. A 10 litre integrated water sample (0-5 m) is now collected using a tube sampler. Each sample is poured through a 10 μm phytoplankton net, and the concentrated sample is then filtered through a 0.8 μm cellulose nitrate filter membrane and the retained material resuspended in 10 mL of filtered seawater. A sub-sample is then examined on a Palmer-Maloney nanoplankton counter and all taxa are identified and enumerated. No net samples are currently considered necessary due to the greatly improved detection limit of 74 cells per litre applicable to the present method of water sample analysis.

Although not documented in the 1999 Operations Manual, monthly sampling was also conducted in King George Sound (KGS-3) from May 1999 until June 2000 when aquaculture operations ceased at the single large southerly lease area. Biotoxin monitoring has not yet commenced at the Mistaken Island area in King George Sound. However, bimonthly sampling started in February 2001 at the Southern Flats area in Cockburn Sound.

The bimonthly field sampling is conducted by the shellfish industry; one set of samples each month is collected under the supervision of Fisheries WA officers.

Biotoxin Monitoring

No routine biotoxin analyses are conducted on shellfish from any aquaculture area. However, mussel samples are collected bi-monthly at the time of phytoplankton sampling and are stored frozen. The samples are stored for six months on a rotational basis, which means that the last twelve mussel samples collected for each area are always available for toxin testing if required.

The most recently collected mussel samples are only analysed when phytoplankton monitoring indicates that a potentially toxic algal species is present with a cell concentration exceeding a "threshold level" prescribed for the relevant species (Table 15). The list of potentially toxic species and their threshold levels have been directly adopted from for the 'New Zealand Marine Biotoxin Management Plan'. Many of the species have not yet been observed in Western Australian marine waters. The type of toxin analysis undertaken depends on the particular toxic algal species present at the time. Shellfish harvesting is allowed to continue while waiting for toxin results, provided that the cell concentration of the species of concern is not excessively above the threshold level and is not known to be toxic in Western Australian waters.

Threshold or trigger levels for potentially toxic species are provided in Appendix 4 of the 'Operations Manual' and are reproduced here in Table 15 for convenience in changed format.

Table 15. WASQAP Phytoplankton action levels

Phytoplankton Species	Concentration to initiate flesh testing
Alexandrium spp *A. acatenella,* *A. catenella,* *A. cohorticula,* *A. fundyense,* *A. lusitanicum,* *A. minutum,* *A. ostenfeldii,* *A. tamarense* *A. tamiyavanichi.*	100 cells/L
Dinophysis spp. *D. acuta,* *D. fortii* *D. norvegica*	500 cells/L
Dinophysis acuminata	1,000 cells/L
Gymnodinium spp. *G. breve,* *G. breve-like,* *G. catenatum* *G. mikimotoi.*	1,000 cells/L
Prorocentrum spp. *P. lima,* *P. mexicanum* *P. minimum*.*	500 cells/L
Pseudo-nitzschia spp. *P. australis,* *P. delicatissima,* *P. fraudulenta,* *P. pseudodelicatissima,* *P. pungens* *P. turgidula*.	5,000 cells/L if >50% of total phytoplankton and 50,000 cells/L if <50% of total phytoplankton

* Added following discovery in Western Australian waters.

Closure and Re-opening Criteria

Closure Criteria

Aquaculture areas are closed for harvesting when the concentration of an algal biotoxin in mussel tissue exceeds the "threshold level" for the relevant toxin as provided in Table 16.

Table 16. WASQAP regulatory flesh closure levels.

Biotoxin Type	WA Threshold Levels*	ANZFA Regulatory Limits
Paralytic Shellfish Poison (PSP)	20 µg saxitoxin equivalent/100 g of edible shellfish flesh	80 µg saxitoxin equivalent/100 g of edible shellfish flesh
Neurotoxic Shellfish Poison (NSP)	200 mouse units (MU)/kg of edible shellfish flesh	200 mouse units (MU)/kg of edible shellfish flesh
Amnesic Shellfish Poison (ASP)	0.2 mg domoic acid equivalent/kg edible shellfish flesh	20 mg domoic acid equivalent/kg edible shellfish flesh
Diarrhetic Shellfish Poison (DSP)	0.2 mg/kg edible shellfish flesh	0.2 mg okadaic acid equivalent/kg edible shellfish flesh

*The threshold levels provided for PSP and ASP toxins are lower than the corresponding regulatory limits specified by ANZFA in the Australian New Zealand 'Food Standards Code'.

There are no closure criteria based on the cell concentration of a toxic algal species exceeding a prescribed abundance, pending the results of toxin testing of mussel meat. Similarly, there are no criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

The Executive Director of Public Health may by order prohibit the cultivation or harvesting of food generally, or food of a specified class or description, in or from a specified area if he is of the opinion that the food may be dangerous or injurious to persons who consume the food [Health Act 1911, Part VIII, Division 4, Section 246W].

In addition, Regulation 69 (h) of the Fish Resources Management Regulations 1995 (relating to conditions of an aquaculture licence) states that the holder of a licence must ensure that fish, which the licence holder is aware or suspects is contaminated, is not removed from the aquaculture site without the prior written permission of the Executive Director.

Re-opening Criteria

The need for appropriate toxin testing of mussel samples before a closed aquaculture area may be re-opened is clearly identified; however, on reading the 'Operations Manual' there is considerable confusion as to exactly what sampling and testing is required. Subsequent clarification revealed that in the case of PSP toxins three consecutive "clear" samples, collected over a minimum period of 14 days, are necessary. Three consecutive "clear" samples collected over a minimum period of 10 days are necessary following shellfish contamination by ASP, DSP or NSP toxins.

There are no specific re-opening criteria specified in the 'Operations Manual'. However, reference to final samples needing to be "clear" means that biotoxin concentrations in the three consecutive mussel samples must be lower than the prescribed toxin "threshold levels" (Table 16).

No criteria are provided based on the absence or reduction in abundance of the causative toxic algal species to cell concentrations below a prescribed abundance, or criteria based on the absence of any shellfish poisoning reported since the date of the first "clear" biotoxin sample.

Program Administration

The DHWA and FWA are jointly responsible for the administration of the WASQAP and have signed a joint Memorandum of Understanding with the Australian Quarantine and Inspection Service (AQIS) concerning the sanitary control (including marine biotoxin control) of fresh and frozen molluscan shellfish intended for exportation from Western Australia. By complying with the requirements of the Australian Shellfish Quality Assurance Program (ASQAP) both domestic and export requirements are satisfied. The Memorandum of Understanding and the Operations Manual of the WASQAP document the responsibilities of the two key government agencies.

The aquaculture industry fully supports and plays an active role in the operation of the biotoxin management program. The *Fish Resources Management Act 1994* imposes certain conditions on aquaculture licenses to ensure the active involvement of industry members in the quality assurance program. License holders must establish and maintain at all time a "health and quality" assurance program for all shellstock produced. Breaching this condition may result in the cancellation, failure to renew or suspension of the aquaculture license.

Western Australia also has a 'Hazardous Algal Blooms Committee', which has broad representation from all relevant government agencies including DHWA (Toxicology and Food Safety Sections of Environmental Health Services), Fisheries WA, Waters and Rivers Commission (WRC), Environmental Protection Authority, Water Authority and Department of Agriculture. Dr. Jane

Latchford (WRC) currently chairs the Committee. The WRC is responsible for algal bloom management in rivers and estuaries and they undertake regular water sampling to monitor waterways in Western Australia. The Commission notifies DHWA whenever an algal bloom has been assessed as potentially hazardous.

The procedures to be followed in the event of a toxic algal bloom at an aquaculture zone are described in the Operations Manual of the WASQAP. Recommendations to cease harvesting or to formally close a harvesting area are made by the WASQAP Manager (HDWA), who is also responsible for notifying industry representatives and all relevant government agencies. More detailed procedures and guidelines are provided in a more general 'Draft Algal Bloom Notification and Response Plan' prepared by DHWA. Improvements to the closure and re-opening procedures in the Operations Manual should occur following completion of the Plan.

Fisheries WA are responsible for all formal closures of harvesting areas based on closure recommendations made by HDWA. Fisheries Officers then immediately inspect the relevant areas to ensure that no illegal harvesting is occurring and regularly monitor closed areas from the time of closure to re-opening.

Internal Review
It is intended to review the 'Operations Manual' of the WASQAP at least annually with amendments being adopted on an as-needs basis. An "amendment history" section is planned.

5.8.1 Key Strengths of WASQAP Biotoxin Management Plan

(i) The risk of shellfish poisoning appears to be comparatively low, vis-à-vis most other States. No aquaculture area closures have been necessary in marine waters to date.
(ii) The surveillance strategy is based primarily on bimonthly phytoplankton monitoring combined with relevant biotoxin testing when potentially toxic algal species are present above a specified 'threshold level'.
(iii) Quantitative data to species level is provided, with a low detection limit.
(iv) DHWA and FWA are jointly responsible for administration of WASQAP, with additional bloom management support provided by Western Australian 'Hazardous Algal Blooms Committee'.
(v) Strong legislative powers are in place to ensure the active involvement of the aquaculture industry in marine biotoxin monitoring, and to restrict or prevent shellfish harvesting if the quality of water is, or is likely to become, a threat to public health.

5.8.2 Key Weaknesses in WASQAP Biotoxin Management Plan

(i) *Insufficient annual funding is available to the WASQAP Manager to conduct routine phytoplankton monitoring at all aquaculture areas and to conduct necessary biotoxin analyses.*
Industry funding in 2000/2001 is only about $5,000-$7,500 which covers the costs of bimonthly phytoplankton monitoring at the Kwinana Grain Terminal site in Cockburn Sound and at Albany in Oyster Harbour. Additional industry and government funding is required to pay for the bimonthly monitoring recently started at Southern Flats in Cockburn Sound, and to initiate bimonthly monitoring at the Mistaken Island aquaculture area in King George Sound. Contingency funding should also be provided to investigate the development of all potentially toxic algal blooms. Research funding is also required to conduct strategic biotoxin analyses of algal and shellfish samples to determine which algal species produce which toxins, and to determine appropriate 'threshold levels' for all potentially toxic algal species in Western Australian waters.

(ii) *Inadequate public health protection from potential biotoxin contamination of commercially or recreationally harvested wildstock shellfish resources is in place.*

Biotoxin surveillance conducted by the WASQAP primarily concerns the safety of commercially cultured mussels and oysters grown in designated aquaculture areas. However, no biotoxin testing has been conducted on commercial scallops. 'Roe-on' and whole body tissue scallops, as sold directly to restaurants and retail outlets, are at potentially higher risk from contamination with marine biotoxins than muscle only product, but all scallops should be tested. The potential for pearl oyster meat sold for human consumption to be contaminated by biotoxins should also be investigated. Furthermore, no routine phytoplankton monitoring occurs in wild harvest shellfish areas where the public may collect mussels, oysters, pipis, clams, razorfish, etc. along the shoreline. Although no public health alerts concerning toxic marine algal blooms have been necessary in Western Australia to date, this situation may change in the future. DSP toxins have been found in shellfish in harvesting area waters. Ballast water introductions of toxic algal species originating from other Australian States and/or overseas countries may also occur at any time. Cockburn Sound and Albany are higher-risk areas because of shipping movements and port facilities, and shellfish poisoning resulting from recreational shellfish harvesting could do much damage to the shellfish culture industry in these areas.

(iii) *Key elements and considerable detail concerning biotoxin management are lacking in the current WASQAP 'Operations Manual'.*

Possibly because there has been no experience of toxic algal blooms in any aquaculture area in the State to date, and no public health alerts or harvesting area closures have been necessary, insufficient attention has been given to the issue of biotoxin management in the current 'Operations Manual'. Section 11 – "Contingency Plan for the Control of Algal Blooms" – consists of only three lines making reference to two other sub-sections and one of the appendices. Either a separate comprehensive 'Biotoxin Management Plan' should be prepared, as in some other States, or a comprehensive biotoxin section should be presented in a substantially revised 'Operations Manual'. Although the review team has some confidence in the biotoxin surveillance arrangements currently operating in three of the four aquaculture areas in Western Australia, based on recent verbal and written communications, no such positive assessment can be gained by reading the present manual. Consideration should be given to all components outlined in the "suggested contingency plan for the control of marine biotoxins" contained in the 'Operations Manual' of the Australian Shellfish Sanitation Control Program. Greater detail is needed concerning sampling sites, phytoplankton sampling, methods of phytoplankton sample analysis, closure and re-opening criteria (and guidelines for their application), phytoplankton and biotoxin "threshold levels", etc.

(iv) *No public health protection is provided for consumers of cultured mussels harvested from Mistaken Island aquaculture area.*

No routine phytoplankton monitoring or biotoxin testing is currently conducted at the Mistaken Island aquaculture area in King George Sound. This growing area should be included in the biotoxin surveillance program.

(v) *The toxicity of many potentially toxic algal species present in West Australian coastal waters is currently unknown, and the "threshold levels" for known toxic species should be determined for local environmental conditions.*

DSP toxins (determined by ELISA) have been detected in mussel samples collected on twelve occasions throughout 2000 when *Dinophysis acuminata*, *Prorocentrum lima* and/or *P. mexicanum* were present in reasonable numbers. Relatively low okadaic acid but high pectenotoxin concentrations were also found in one mussel sample collected in September 2000, analysed by Queensland Health Services using LC-MS, when *D. acuminata* was abundant. Further testing is required to determine exactly what toxins are produced by each

potentially toxic phytoplankton species. HPLC analysis of algal samples is also required to establish which Western Australian species of *Pseudo-nitzschia* produce domoic acid. Appropriate threshold levels relevant for local conditions will then be able to be set.

(vi) *Incorrect biotoxin "threshold levels" have been adopted for PSP and ASP toxins, and additional closure criteria are necessary to ensure adequate public health protection.*

Regulatory limits specified by ANZFA in the Australian New Zealand 'Food Standards Code' should be used for all toxin types. The standard for PSP toxins is 0.8 mg saxitoxin equivalent/kg (or 80 μg/100 g as commonly used), and for ASP toxins is 20 mg domoic acid equivalent/ kg (or 20 μg/g). Toxin "threshold levels" adopted for PSP and ASP toxins in the current 'Operations Manual' are lower than their corresponding ANZFA standards, and their application could trigger inappropriate biotoxin testing and harvesting area closures. The DSP threshold levels should be expressed in terms of okadaic acid equivalence. Additional closure criteria are required in addition to the present criteria, which states that aquaculture areas must be closed for harvesting when toxin concentrations in mussel meat exceed the relevant threshold levels. There are no closure criteria based on the cell concentration of a toxic algal species exceeding a prescribed abundance limit, pending the results of toxin testing of mussel meat. There are also no criteria based on the reporting of human illness fitting the case definitions for PSP, ASP, DSP or NSP.

(vii) *The present re-opening criteria require considerable clarification and amendment, and additional re-opening criteria are required.*

The current section on re-opening criteria is poorly worded and requires substantial amendment. Firstly, it should be clearly stated that the re-opening of a previously closed aquaculture area can only occur when certain toxin criteria are satisfied. This is only implied through use of the term "clear" samples. For all toxins, concentrations should be below the relevant regulatory limit in three consecutive samples collected over a minimum period of 14 days. Additional criteria should be provided based on the absence or reduction in abundance of the causative toxic algal species to cell concentrations below prescribed threshold or 'action' levels, and criteria based on the absence of any shellfish poisoning reported since the date of the first "clearance" sample. Guidelines concerning the application of the criteria should also be documented.

(viii) *Annual reviews of the WASQAP Operations Manual are required.*

The current biotoxin surveillance component of the Operations Manual is out of date, but is under review and the revised 2001 Operations Manual will be issued at the end of June 2001. Annual reviews of the Operations Manual should be conducted as originally planned. Such reviews are needed to re-assess the phytoplankton and biotoxin monitoring strategy and to evaluate the efficacy of biotoxin management controls given a further 12 months' data and information.

5.9 Summary

The following general issues relate to most of the current State and Territory Shellfish Quality Assurance Programs.

- **Funding for routine monitoring** - In general funding levels for routine monitoring appear low. In some instances, programs are funded in part by industry and part by government, but in general the level needs to be raised. Internationally, approximately 1-2% of the value of the industry is spent on biotoxin monitoring. Nationally in Australia it is approximately 0.01-0.02% of the value of the industry.
- **Funding for contingency monitoring** - There is little in the way of funding available for contingency plans. In the event of a major marine biotoxin outbreak, resources would be stretched.
- **What species produce what?** Strategic research needs to be undertaken to find out which species produce which toxins in which States. Until species and multiple strains of species have been tested, monitoring programs can only be based on the best available Australian and International information which may not be correct for particular areas. Continuing on from this, more monitoring ('research') will allow the setting of appropriate and relevant action levels for potentially toxic phytoplankton species.
- **Frequency of sampling** – The frequency of sampling for a long-term monitoring program is a compromise between the available resources (money and people), the history of an area, and what is ideally required. In areas where there is a long-term history of phytoplankton monitoring or biotoxin testing, with no positive results, then the testing frequency for those toxins may be less. However, it is very important to keep in mind the other potential species and toxins that may be present, and for which there may not be as much information available.
- **Frequency of phytoplankton sampling** - It is encouraging to see the amount of phytoplankton monitoring that is carried out. However in general, the sampling is carried out too infrequently. Phytoplankton population dynamics can change within a short period of time, in some instances days. As part of an on-going monitoring program, sampling ideally should be carried out weekly, but this may be affected by the availability of resources or local conditions or history (see previous bullet point). Monthly sampling is generally too infrequent, as events ('blooms') could occur in between sampling.
- **Types of phytoplankton sampling** - Quantitative bottle or tube sampling for phytoplankton should be used more than qualitative net tow sampling. Net tows are an excellent way in which to concentrate cells to test for toxins, and initiate cultures, but are not ideal for use in monitoring programs. Nets can cause damage to fragile cells such as *Karenia* cf *brevis (=Gymnodinium* cf *brev*e), and *Karenia mikimotoi (=Gymnodinium mikimotoi)* types, as well as some of the smaller species. This means that the samples may not give an accurate indication of the true structure of the phytoplankton community hence there is a risk of false negatives. Bottle or tube samples giving quantitative results are required in order to use phytoplankton monitoring successfully as an early warning system, because levels can be set at which actions such as triggering flesh testing can be made.
- **Recreational gathering** – Recreational gathering is not included in the scope of this report, but there is significant overlap in some areas of both commercial and recreational activities. Wild harvest shellfish are affected by marine biotoxins just as cultured shellfish are, and should be included in management plans. Illness caused by recreationally gathered shellfish impacts on the image of commercial shellfish safety, and it is in the interests of all shellfish industries to have monitoring programs in place for recreational gathering.
- **Commercial wild harvest shellfish** - These are generally not included in monitoring plans. Wild harvest shellfish are affected by marine biotoxins just as cultured shellfish are, and should

be included in management plans. Illness caused by wild harvest shellfish (such as pipis and scallops) impacts on the image of all shellfish safety. It is in the interests of the all commercial shellfish industries to have monitoring programs in place for wild harvesting of shellfish.

- **Closure criteria** – In most cases a criterion based on illness needs to be added. For example: "Cases of human illness consistent with the case definitions for PSP, ASP, NSP or DSP have resulted from the consumption of shellfish from (a particular area)".
- **Re-opening criteria** – In most cases a criteria needs to be added that "No cases of human illness fitting the case definitions for PSP, ASP, NSP or DSP have resulted from the consumption of shellfish harvested since the date of collection of the first clearance sample from within or adjacent to the closed area".
- **Review** – Plans need to be reviewed annually and amendments included so that the current operating procedure is recorded at all times.

Thanks are due to the individual State and Territory Program Managers for their co-operation and assistance and for the provision of relevant biotoxin documents. The Program Managers are a dedicated group of people, who have broad and onerous responsibilities concerning shellfish quality assurance, and the present review could not have occurred without their full co-operation. Any deficiencies found in the current plans are primarily due, not to them, but to policy weaknesses and limited funding and manpower resources that prevent the Program Managers from running a comprehensive program covering all potential risks.

6 REVIEW OF EXISTING ANALYTICAL LABORATORY SERVICES

6.1 Introduction

This section is a review of the analytical services relevant to marine biotoxin monitoring available in Australia. Information was collected by questionnaires (Appendices 2 and 3) which were distributed to known labs, and through the ARNAT network of researchers working on algal toxins. There were no responses received from state government employees who undertake analysis as part of their responsibilities.

Five laboratories offering services in marine microalgae identification and seven laboratories offering services in marine biotoxin analysis responded to the questionnaire. This is by no means an exhaustive list of relevant analytical services available in Australia. ARNAT has compiled a list of researchers and commercial labs known to be working on harmful microalgae and/or biotoxins (see the ARNAT website at www.aims.gov.au/arnat).

6.2 Overview of Laboratory/Analytical Requirements

In order to run a marine biotoxin monitoring program that meets international requirements (section 2 of this report) and uses internationally accepted methods (see section 3 of this report), there are three main analytical services that need to be available.
The three types of testing required are:

i) marine microalgae identification and enumeration of seawater samples;
ii) testing shellfish samples by mouse bioassay (for PSP, NSP, DSP);
iii) testing shellfish samples by instrument (e.g. HPLC for ASP, LC-MS for PSP, DSP and NSP).

Each will be discussed in turn as to specific requirements.

6.2.1 Marine Microalgae Identification and Enumeration

A laboratory performing this type of testing needs to have a variety of microscopes, preferably inverted, which are used for routine analysis. They also require access to a more high-powered compound microscope with epi-fluorescence capability, or an electron microscope, in order to confirm the identification of species that can be difficult to identify confidently using routine light microscope methods (e.g. *Pseudo-nitzschia* and *Alexandrium* species). Identification of some difficult genera can be done using staining techniques (e.g. Calcofluor) or genetic techniques (e.g. whole cell DNA probes).

The analysts performing this work should have extensive training in this field, either as part of research work or by attending training courses (e.g. UNESCO-IOC courses). They should also have ready access to experts, who can give definitive answers on species identifications.

The laboratory needs to offer fast turnaround times, as results are required urgently in order for phytoplankton monitoring to act successfully as an early warning for potential biotoxin contamination in bivalve shellfish.

6.2.2 Testing Shellfish by Mouse Bioassay (for PSP, NSP, DSP)

There are two separate parts to the testing by mouse bioassay, which do not necessarily have to be performed in the same laboratory. The first part of the testing requires organic chemists for the solvent extraction of the toxins; extracts are then injected into the mice.

A laboratory performing this type of work needs to have general 'wet' chemistry facilities, suitable for performing extractions of shellfish. The laboratory also requires a mouse breeding facility (or access to one) that is capable of increased capacity in the event of a widespread toxin outbreak. The analysts performing this work need to have experience in observing the symptoms of mouse death for a variety of toxins, as this determines the type of toxin causing death.

6.2.3 Shellfish Testing by Instrument (e.g. HPLC for ASP, LC-MS for PSP, DSP, NSP)

A laboratory performing shellfish testing by instrument requires analysts with experience in organic chemistry, operation of the instruments, and knowledge for trouble-shooting if necessary. The lab also needs to have the ability to increase capacity in the event of a widespread toxin outbreak.

While the use of HPLC for the determination of ASP is well documented, the use of LC-MS technology for PSP, DSP, NSP and other toxins is in the method development phase.

6.2.4 Requirements for All Testing Facilities

Ideally testing facilities would be run as commercial laboratories, which have the necessary links with research scientists who offer advice when required, undertake research and in general assist in the testing if needed. The laboratory should comply with the ISO/IEC17025 standard "General requirements for the competence of testing and calibration laboratories." This International standard is for use by laboratories in developing the quality, administrative and technical systems that govern their operations.

All testing facilities should hold National Associated Testing Authorities, Australia (NATA) accreditation for the particular methods they offer. In the case of microalgae testing, there needs to be a clear differentiation between accreditation for freshwater and marine microalgae analysis, as these fields do not easily overlap. For export product, laboratories may also be required to hold USFDA accreditation.

All labs need to participate in proficiency testing or inter-laboratory collaborative testing, and analysts should participate in on going training to maintain their proficiency.

Labs need to have a data management system that allows the secure storage and ready availability of data for information purposes.

6.3 Analytical Services Currently Available in Australia

6.3.1 Marine Microalgae Identification and Enumeration

See Appendix 4 for a summary of micro-algal testing services available in Australia.

Of the organisations that responded to the questionnaire, one laboratory specialises in the analysis of marine microalgae. This lab has two experts performing this analysis; they have several cell concentration methods available depending on client requirements, use a variety of microscopes and have both fluorescence and electron microscopy available for confirmatory methods. Two of the labs also offer expertise in freshwater algae analysis. One of the labs is quite specific about the type of work they do, i.e. they analyse ballast water and sediment for toxic dinoflagellates (i.e. *Alexandrium* species and *Gymnodinium catenatum*). Generally the labs offer a quick turnaround on analysis, (i.e. <24 hours), and in general they are working below their sample capacity.

Only one lab has NATA accreditation, although several others indicated in their responses that they are working towards gaining accreditation in the near future.

6.3.2 Shellfish Testing by Mouse Bioassay and Instrument

See Appendix 5 for a summary of shellfish flesh testing services available in Australia.

Of the organisations responding to the questionnaire, five laboratories offer services on a commercial basis, three perform research into marine biotoxins, and two undertake both commercial and research work into marine biotoxins.

One laboratory has NATA accreditation for paralytic shellfish toxin analysis, and one lab has NATA accreditation for domoic acid analysis. Several other labs have NATA accreditation for other services they offer.

PSP testing: Four laboratories offer commercial testing for PSP toxins. Only one is by the internationally (i.e. EU, USFDA) accepted AOAC mouse bioassay method, for which the laboratory holds NATA accreditation. The remaining three use HPLC methods.

DSP testing: Two laboratories offer testing for DSP toxins. One is by screen only (i.e. there is no confirmation of compounds) and it is rarely performed. This screen test method is not specific to DSP toxins, but rather is a screen for lipid soluble toxins such as the DSP and NSP toxins, and may also react to a variety of other substances such as free fatty acids. The other laboratory offering DSP testing does so by LC-MS, which enables specific compounds to be identified and quantified.

ASP testing: Two laboratories offer commercial testing for ASP toxins. One laboratory has NATA accreditation for this test. Both use HPLC-UV as the testing method.

NSP testing: No commercial testing is available for NSP toxins. The lipid soluble toxin screen method can detect NSP toxins, but it also reacts to DSP toxins and may react to a variety of other substances such as free fatty acids.

Other toxins: None of the other toxins (e.g. AZP, Spirolides, etc) have commercially available tests in Australia. Many of these compounds are relatively new and have only been found in one country. Research is being conducted internationally to understand more about these compounds.

Ciguatoxin: Ciguatoxin has not been included in the scope of this review, however it deserves mention here in that there is no commercially available test method for this toxin, although there are research methods available.

The turn-around times offered by the commercial labs are generally fast, and would be suitable as part of a monitoring program.

6.4 Limitations in Analytical Services Currently Available in Australia

6.4.1 Marine Microalgae Identification and Enumeration

In general, many labs offering marine microalgae identification have expanded to offer this service in addition to existing freshwater identification work. Although these two services are obviously related, there is no direct cross-over of taxonomic skills between the two. There is a need for a nation-wide proficiency testing program (inter-laboratory) between all laboratories offering this service, and also between state government employees who are involved in marine microalgae analysis. This program could also include New Zealand laboratories, where analysts need to be able to identify all potentially toxic species.

Analysts should regularly attend taxonomy courses, which are organised from time to time by various organisations. This is important as a means of keeping up to date with name changes, and also as a proficiency exercise.

Some laboratories are accredited by NATA, however there needs to be clear differentiation between accreditation for freshwater and marine algae monitoring.

6.4.2 Shellfish Testing

There is currently no single organisation capable of performing a variety of biotoxin analyses. For example, if a shellfish grower or Quality Program organiser wanted samples tested for PSP, ASP and DSP, they could potentially be in the position of having to send three separate samples to three different labs in three different states. Obviously in terms of running a management program, this would entail high freight costs and delays in the availability of results due to freighting time.

There is currently no laboratory dedicated to solely testing marine biotoxins. Many of the labs offering these services do so as part of an extensive selection of analyses available to clients. It is unclear as to whether there is inter-laboratory testing carried out between the labs performing these tests.

There are only two labs accredited by NATA for biotoxin testing. Many of the other labs hold NATA accreditation for other services they offer, but users need to have clear understanding that accreditation of an organisation does not automatically cover all tests they perform.

6.5 Opportunities for Improvement in Analytical Services Currently Available in Australia

The availability of services relating to marine microalgae and marine biotoxin analysis could be greatly improved by having a “Centre of Excellence” whose main focus is on analysis of water and shellfish for the shellfish industry. By having one (or more) laboratory/ies capable of all related analysis, it would be easier and more economical for clients to have samples freighted, analysed and results reported to them. Given the distances that samples potentially would be required to be freighted, it would make sense to have more than one facility with the expertise and resources to be able to set this up.

Internationally research is being conducted into new tests, which will be quicker and cheaper to run than currently accepted methods. There is value in the promotion and support of Australian researchers becoming involved in testing trials of new methods and in general conducting research into the particular toxins that affect Australian shellfish. This plays a major role in ensuring the program is kept up to date.

Proficiency programs (or inter-laboratory comparison programs) should be set up for both marine microalgae testing and for biotoxin analysis.

7 ORGANISMS POSING A BIOTOXIN THREAT AND THOSE INDUSTRIES AT RISK

7.1 Introduction

Toxic microalgae contaminate shellfish with a variety of biotoxins, which can result in economic losses for the shellfish industry and in some cases result in human health problems. Harmful algal blooms (HABs), some of which also cause the death of shellfish or finfish, appear to be on the increase world-wide, and as aquaculture increases globally so must monitoring for biotoxin producers. Most of the currently described marine biotoxin producing species occur in Australia, and many of those that haven't been recorded will no doubt be observed as monitoring increases. There is also a real risk of new species being introduced via ballast water or translocation of aquaculture products.

The toxic microalgae can be found in temperate and/or tropical coastal waters, with a few species restricted to estuarine environments. However, bodies of water containing blooms of estuarine species can be translocated from estuaries to coastal shellfish beds or marine farms following heavy rainfall.

HAB is a common term used internationally, but it should be noted that while most toxic microalgae do cause problems at bloom concentrations (often observable as colouration of the seawater), there are species that produce toxins in concentrations potentially fatal to humans at extremely low cell numbers per litre of seawater.

Risk assessments can be generated directly from phytoplankton monitoring data, and predictive models are being developed, based on a combination of environmental, nutrient and phytoplankton data. The presence of dinoflagellate cyst beds in sediments, for example *Gymnodinium catenatum*, needs to be assessed so that the risk of mass hatching under suitable conditions can also be predicted. For all these reasons, monitoring regimes must incorporate different sampling protocols and "risk levels" for each species.

The experience in New Zealand has been that all temperate biotoxin producing microalgae genera have been detected and most of the tropical genera have been found in the sub-tropical northern waters. The Australian coastline encompasses all climate zones and it is to be expected that all known biotoxin producing species will be detected over time, and that they will bloom as conditions become favourable to them.

7.2 Microalgae Posing a Biotoxin Threat to Shellfish in Australian Waters

Most toxic microalgae have apparent global distributions, and it is only a matter of time before Australia has most of the known temperate and tropical genera in the coastal and estuarine waters. Australia has already had significant HAB "events", for example the recent contamination of New South Wales' pipis by pectenotoxins.

7.2.1 Identification

Many of the toxic microalgae that cause the various shellfish biotoxins are now well described and their impacts on shellfish and their human health effects are documented, although further toxicological research is required for some bioactive compounds (for example, gymnodimine and yessotoxin). "Algal toxins in Australian and New Zealand seafood products: review of their occurrence, analytical detection and public health implications" (report for the Australia New Zealand Food Authority (ANZFA), July 1998, updated July 2000, by Gustaaf Hallegraeff) is a useful resource. The biennial international Harmful Algal Bloom symposia have contributed

hugely to the dissemination of microalgal related information, and published proceedings of these meetings are also available.

Methods for monitoring microalgae and published descriptions and illustrations of toxic species are presented in the "Manual on Harmful Microalgae" (Hallegraeff *et al.* 1995), an updated edition of which is in preparation, and in the "Aquaculturists' Guide to Harmful Australian Microalgae" (Hallegraeff 1991). An excellent identification manual (Tomas 1997), software training packages (for example, the "Atlas of Dinoflagellates" produced on CD ROM by the Intergovernmental Oceanographic Commission), and several useful "web" sites are easily accessible to assist in the setting up of phytoplankton monitoring programs. International training courses in taxonomic identifications and biotoxin methodologies are also offered regularly (for example taxonomy courses sponsored by the IOC).

7.2.2 Issues

The global distribution and the favoured environments for growth, of toxic microalgae are also well documented, which makes predictions of occurrence of the various species in Australian waters more promising. However, some of the toxic species produce different toxin concentrations per cell depending on the particular environmental and nutrient conditions. Even the daily light/dark changes, the life cycle stage, the associated bacterial flora, or the growth phase of a bloom can result in different toxin concentrations, and to add to these complexities, a known toxic species might also have non-toxic strains. Phytoplankton monitoring programs therefore tend to be conservative, meaning that they are designed to give risk assessments for the greatest potential toxicity in shellfish, and therefore have an assured safety margin.

Additional assays can refine risk assessments, for example DNA probes are used commercially in New Zealand to determine whether a *Pseudo-nitzschia* bloom, identified initially to genus level under the light microscope, is comprised of toxic or non-toxic species, and Calcofluor stains are regularly used in conjunction with UV microscopy to aid in the definitive identification of *Alexandrium* species.

7.3 Shellfisheries Impacted

The identification of Australian shellfish industries at risk from biotoxin producers was collated through analysis of a literature review, and through a questionnaire sent to all State Quality Assurance Program Leaders (see Appendix 1).

7.3.1 At Risk Bivalve Shellfisheries (Cultured and/or Harvested)

Blue mussel (*Mytilus edulis*); WA, SA, VIC, NSW, TAS
Clams (*Venerupis* sp.); TAS
Cockles (Sydney: *Anadara trapezius*, Sand: *Katylesia* sp.); NSW, TAS
Doughboy scallops (*Chlamys asperrimus*); VIC, NSW, TAS
Giant clams (*Tridacna gigas*); QLD
Native flat oyster (*Ostrea angasi*); WA, SA, VIC, NSW, TAS
Pacific oyster (*Crassostrea gigas*); SA, NSW, TAS
Pearl oyster (*Pinctada maxima*); QLD, NT, WA, SA, NSW
Pipi (eg. *Plebidonax deltoides* – at least 7 species); SA, NSW, TAS
Queen scallops (*Equichlamys bifrons*); SA, TAS
Razorfish (*Pinna bicolor*); WA, SA
Saucer scallop (*Amusium* spp.); QLD, NT, WA, NSW
Southern scallop (*Pecten fumatus*); WA, VIC, NSW, TAS
Surf clam (eg. *Dosinia caerulea* – >20 species); NSW

Sydney rock oyster (*Saccostrea glomerata*); QLD, NSW
(NB: all fisheries, whether major, minor or being experimentally trialled, are included above).

7.4 Biotoxin Producing Species Present or Likely to be Present in Australian Waters Sorted by Biotoxin Group

The following list of organisms that pose a threat to Australian shellfisheries was compiled from records of species previously recorded in Australian waters, species common in New Zealand waters (New Zealand industry and Ministry of Health phytoplankton records), and species with otherwise global distributions (Hallegraeff *et al.* 1995).

7.4.1 Domoic Acid (DA) Producers

Domoic acid causes amnesic shellfish poisoning (ASP). All the microalgae involved are diatoms.

Highly toxic species:
Pseudo-nitzschia australis (confirmed toxic strains in Australia),
P. multiseries,
P. pungens (usually non-toxic, but toxic strains produce high concentrations of domoic acid per cell).

Low to moderate toxicity, with non-toxic strains also occurring:
P. turgidula,
P. pseudodelicatissima,
P. delicatissima,
P. fraudulenta
Nitzschia navis-varingica (domoic acid was recently confirmed for an isolate from brackish Vietnamese waters).

Toxicity uncertain:
P. subpacifica,
P. lineola,
P. subfraudulenta,
P. cuspidata.

Non-toxic:
P. multistriata,
P. heimii.

Shellfish industries most at risk
Scallop and Blue mussel. Scallops in particular have been recorded with high concentrations of domoic acid in the gut (>600 ppm), but all shellfish can be contaminated.

States likely to be impacted
All states (although toxicity in tropical waters is unclear).

Useful references for identification of organisms causing ASP:

Hallegraeff, G. M. 1994: Species of the diatom genus *Pseudonitzschia* in Australian waters. *Botanica marina* 37: 397-411.

Hasle, G. R. and Fryxell, G. A. 1995: Taxonomy of diatoms. In: Hallegraeff, G. M.; Anderson, D. M.; Cembella, A. D. (eds.), Manual on Harmful Marine Microalgae. IOC Manuals and Guides No.33 UNESCO, pp.339-364.

Hasle, G. R., Lange, C. B. and Syvertsen, E. E. 1996: A review of *Pseudo-nitzschia*, with special reference to the Skagerrak, North Atlantic, and adjacent waters. *Helgoländer meeresunters 50*: 131-175.

Hasle, G. R. and Syvertsen, E. E. 1997: Marine diatoms. In: Tomas, C. R. (ed.), Identifying marine diatoms and dinoflagellates. Academic Press, San Diego, CA, pp. 5-386.

Rhodes, L. 1998: Identification of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) in New Zealand coastal waters, using lectins. *New Zealand Journal of Marine and Freshwater Research* 32: 537-544.

Rhodes, L. L., Scholin, C., Garthwaite, I., Haywood, A. and Thomas, A. 1998: Domoic acid producing *Pseudo-nitzschia* species educed by whole cell DNA probe-based and immunochemical assays. In: Reguera, B., Blanco, J., Fernandez, M. L., Wyatt, T. (eds.), Harmful Algae. Xunta de Galicia and IOC of UNESCO. pp. 274-277.

7.4.2 Saxitoxin (STX) Producers (includes STX Derivatives, e.g. Gonyautoxins and C Toxins)

Saxitoxins and derivatives cause paralytic shellfish poisoning (PSP). The microalgae involved are dinoflagellates and cyanophytes (blue-green algae, not covered in this report).

Dinoflagellates:
Gymnodinium catenatum.
Pyrodinium bahamense var. *compressum* (potentially in tropical habitats).
Alexandrium catenella,
A. minutum,
A. ostenfeldii,
A. tamarense (which also has non-toxic strains),
A. angustitabulatum (identified in New Zealand waters).

Non-toxic *Alexandrium* species include:
A. margalefi,
A. pseudogonyaulax,
A. fraterculus.

Blue-greens:
Anabaena circinalis

Shellfish industries at risk
All filter feeding molluscs and their predators are potentially at risk. High STX concentrations can be retained in the siphon, for example in clams.

States likely to be impacted
Tas, Vic, SA, NSW, WA, NT (*Pyrodinium*).

Useful references for identification of organisms causing PSP:

Balech, E. 1985: The genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station Publication, Sherkin Island, Co. Cork, Ireland, pp. 151.

Hallegraeff, G. M. 1991: Aquaculturalists guide to harmful Australian microalgae. Fishing Industry Training Board of Tasmania and CSIRO Division of Fisheries, pp. 111.

Mackenzie, L. and Berkett, N. 1997: Cell morphology and PSP-toxin profiles of *Alexandrium minutum* in the Marlborough Sounds, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 31: 403-409.

Mackenzie, L., White, D., Oshima, Y. and Kapa, J. 1996: The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. *Phycologia* 35: 148-155.

Steidinger, K. A. and K. Tangen. 1997: Dinoflagellates. In: Tomas, C. R. (ed.), Identifying marine diatoms and dinoflagellates. Academic Press, San Diego, CA, pp. 387-584.

Taylor, F. J. R., Fukuyo, Y. and Larsen, J. 1995: Taxonomy of harmful dinoflagellates. In: Hallegraeff, G. M.; Anderson, D. M.; Cembella, A. D. (eds.),. IOC Manuals and Guides No. 33. UNESCO, pp. 283-317.

7.4.3 Brevetoxin (BTX) Producers (includes BTX Derivatives).

Brevetoxins causes neurotoxic shellfish poisoning (NSP). The microalgae responsible include dinoflagellates and raphidophytes (flagellates).

Dinoflagellates:
Karenia (=Gymnodinium) cf *breve*

The following are considered potential BTX producers, although further confirmation is required:
Karenia mikimotoi (=Gymnodinium mikimotoi),
Gymnodinium aureolum,
Karlodinium micrum (=Gymnodinium galatheanum),
Karenia (=Gymnodinium) bidigitatum (found in New Zealand*),*
Gymnodinium impudicum,
Gymnodinium. pulchellum,
Karenia (=Gymnodinium) papilionacea (sp. in edit),
Karenia (=Gymnodinium) selliforme (found in New Zealand) (sp. in edit).

Raphidophytes:
Heterosigma akashiwo,
Chattonella antiqua/marina,
Fibrocapsa japonica.

NB: A recent publication by Daugbjerg *et al.* (2000) has proposed three new genera (*Akashiwo, Karenia* and *Karlodinium*) which would alter the species names for many *Gymnodinium* species.

Shellfish industries at risk
Potentially all shellfish can be contaminated.

States likely to be impacted
WA, SA, Vic, NSW, Tas.

Useful references for identification of organisms causing NSP:

Daugbjerg, N., Hansen, G., Larsen, J. and Moestrup, O. 2000: Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. *Phycologia* 39: 302-317.

Haywood, A. 2001: Systematics of Dinoflagellates of the order Gymnodiniales. Unpublished thesis, NZ School of Biological Sciences, University of Auckland.

Steidinger, K. A. and Tangen, K. 1997: Dinoflagellates. In: Tomas, C. R. Ed. Identifying marine diatoms and dinoflagellates. Academic Press, San Diego, CA, pp. 387-584.

Taylor, F. J. R., Fukuyo, Y. and Larsen, J. 1995: Taxonomy of harmful dinoflagellates. In: Hallegraeff, G. M.; Anderson, D. M.; Cembella, A. D. (ed.), IOC Manuals and Guides No. 33. UNESCO, pp. 283-317.

7.4.4 Diarrhetic Shellfish Toxin (DSP) Producers

Toxins include okadaic acid (OA) and the related dinophysis toxins (DTXs) and their diol esters. A range of microalgae, including planktonic, benthic and epiphytic dinoflagellates, are responsible. Yessotoxin and pectenotoxin and their derivatives are included in this group, although their primary effects are on cardiac muscle and liver respectively. Oral toxicity in mice/rats and the risk to human health has yet to be established for yessotoxins and pectenotoxins.

(i) Producers of okadaic acid, dinophysistoxins and diol esters.
Dinophysis acuta,
D. acuminata,
D. caudata,
D. fortii,
D. hastata,
D. mitra,
D. rotundata,
D. tripos (some stains*),*
Prorocentrum lima, (possibly *P. elegans, P. hoffmannianum, P. concavum.)*

(ii) Yessotoxin and derivatives.
Protoceratium reticulatum (syn: Gonyaulax grindleyi),
Lingulodinium polyedrum (syn: Gonyaulax polyedra; possible producer*),*
Coolia monotis (uncertain).

(iii) Pectenotoxins and derivatives
Pectenotoxins reportedly caused illness in shellfish consumers (pipis) at South Ballina Beach, NSW in December 1997 and at Stockton Beach, NSW in March 1998.
Dinophysis acuminata
D. acuta,
D. fortii.
D. caudata was associated with pectenotoxins in shellfish in Boston and Proper Bays, SA, in August 2000.

(iv) Azaspiracid.
In the EU, this compound is regulated for as a DSP toxin. This toxin has only been detected in shellfish in Ireland to date. The identification of the causative organism has yet to be published.

Shellfish industries at risk
Potentially all shellfish can be contaminated.

States likely to be impacted
All states.

Useful references for identification of organisms causing DSP:

Hallegraeff, G. M. 1991: Aquaculturalists guide to harmful Australian microalgae. Fishing Industry Training Board of Tasmania and CSIRO Division of Fisheries, pp. 111.

Steidinger, K. A. and Tangen, K. 1997: Dinoflagellates. In: Tomas, C. R. (ed.), Identifying marine diatoms and dinoflagellates. Academic Press, San Diego, CA, pp. 387-584.

Suzuki, T., Mackenzie, L., Stirling, D. and Adamson, J. 2001: Pectenotoxin-2 seco acid: a toxin converted from pectenotoxin-2 by the New Zealand Greenshell mussel, *Perna canaliculus. Toxicon* 39: 507-514.

Taylor, F. J. R., Fukuyo, Y. and Larsen, J. 1995: Taxonomy of harmful dinoflagellates. *In*: Hallegraeff, G. M.; Anderson, D. M.; Cembella, A. D. (eds.), IOC Manuals and Guides No. 33. UNESCO, pp. 283-317.

7.4.5 Other Microalgae With Potential Toxic Impacts on Shellfish

Gymnodimine producers:
Gymnodimine producing species have been responsible for the mortalities of a variety of marine fauna in New Zealand's southern waters.

Karenia (=Gymnodinium) selliforme (sp. in edit).

States likely to be impacted:
All states

Palytoxin producers:
Palytoxin, and the related ostreocin and derivatives, pose a potential shellfish toxin problem, although there is no hard evidence of uptake by shellfish to date. Palytoxin causes a neurotoxic shellfish poisoning, and deaths due to eating fish contaminated with this compound have been recorded.

Ostreopsis siamensis.

States likely to be impacted:
QLD, NT, SA, TAS

Cooliatoxin producers:
Coolia monotis

States likely to be impacted:
All states

Spirolide and prorocentrolide producers:
Alexandrium ostenfeldii and *Prorocentrum maculosum* respectively.

Microalgae related to deaths of shellfish:
Blooms of the diatom *Rhizosolenia chunii* have been linked to a bitter taste in shellfish, and have caused shellfish deaths (Hallegraeff 1991).

The heterotrophic dinoflagellates *Pfiesteria piscicida* and *P. shumwayae* have been known to feed voraciously on shellfish larvae, as well as causing massive deaths of fish in the United States. *Pfiesteria* also produces a water soluble compound (not yet characterised) which causes neurological symptoms in humans in contact with blooms. *Pfiesteria shumwayae* has been confirmed in New Zealand waters (Lesley Rhodes, Cawthron Institute, New Zealand, pers. comm.), and also in NSW and Tasmanian waters (Gustaaf Hallegraeff, University of Tasmania, Australia, pers. comm.).
Blooms of *Karenia (=Gymnodinium) selliforme*, the only known gymnodimine producer, have been responsible for massive shellfish mortalities in Southern New Zealand, and *K. (=Gymnodinium) brevisulcatum* was responsible for massive mortalities of marine biota in Wellington Harbour, New Zealand, in 1998 (Chang 1999). That toxin is still being characterised.

Useful references:

Chang, F. H. 1999: *Gymnodinium brevisulcatum* sp. nov. (Gymnodiniales, Dinophyceae), a new species isolated from the 1998 summer toxic bloom in Wellington Harbour, New Zealand. *Phycologia* 38: 377-384.

Hallegraeff, G. M. 1991: Aquaculturalists guide to harmful Australian microalgae. Fishing Industry Training Board of Tasmania and CSIRO Division of Fisheries, pp. 111.

Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. (eds.), 1995: Manual on Harmful Marine Microalgae. IOC Manuals and Guides No.33 UNESCO, 551 pp.

Mackenzie, L., Haywood, A., Adamson, J., Truman, P., Till, D., Seki, T., Satake, M. and Yasumoto, T. 1996: Gymnodimine contamination of shellfish in New Zealand. *Proceedings of the 7th International* conference on toxic marine phytoplankton, Sendai, Japan. In: Harmful and toxic algal blooms. Yasumoto *et al.* (eds.), IOC of UNESCO, pp. 155-158.

Rhodes, L. L. 2000: Report on the symposium on Harmful Algae in the U.S. Woods Hole, December 2000. Report for Ministry of Research, Science and Technology. Cawthron Report No. 614.

Rhodes, L.L., Adamson, J., Suzuki, T., Briggs, L. and Garthwaite, I. 2000: Toxic epiphytic marine dinoflagellates *Ostreopsis siamensis* and *Coolia monotis* (Dinophyceae) in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 34: 371-384.

Steidinger, K. A. and Tangen, K. 1997: Dinoflagellates. *In*: Tomas, C. R. (ed). Identifying marine diatoms and dinoflagellates. Academic Press, San Diego, CA, pp. 387-584.

Taylor, F. J. R., Fukuyo, Y. and Larsen, J. 1995: Taxonomy of harmful dinoflagellates. In: Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. (eds.), IOC Manuals and Guides No. 33. UNESCO, pp. 283-317.

7.5 Biotoxin Producing Species Present or Likely to be Present in Australian Waters Sorted Into Categories

Category A - Species known to be present in Australian waters and proven to produce toxins either in Australia or internationally:

Alexandrium catenella (saxitoxin and derivatives)
Alexandrium minutum (saxitoxin and derivatives)
Alexandrium ostenfeldii (saxitoxin and derivatives, also produces spirolides in Canada)
Alexandrium tamarense (saxitoxin and derivatives, also has non-toxic strains)
Dinophysis acuminata (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis acuta (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis caudata (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis fortii (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis hastata (okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis mitra (okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis rotundata (okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis tripos (some strains produce okadaic acid, dinophysis toxins and diol esters)
Gymnodinium catenatum (saxitoxin and derivatives)
Karenia cf *brevis (=Gymnodinium* cf *breve*) (brevetoxins)
Prorocentrum lima (okadaic acid?, dinophysis toxins? and diol esters?)
Pseudo-nitzschia australis (domoic acid)
Pseudo-nitzschia delicatissima (domoic acid)
Pseudo-nitzschia fraudulenta (domoic acid)
Pseudo-nitzschia multiseries (domoic acid)
Pseudo-nitzschia pseudodelicatissima (domoic acid)
Pseudo-nitzschia pungens (usually non-toxic, but toxic strains produce high concentrations of domoic acid per cell)

Pseudo-nitzschia turgidula (domoic acid)
Pyrodinium bahamense var. *compressum* (in tropical habitats) (saxitoxin and derivatives)

Category B - Potential toxin producing species (ie toxicity untested/unclear) known to be present in Australian coastal waters

Alexandrium pseudogonyaulax (possible STX and derivatives, goniodomin)
Chattonella marina/antiqua (possible brevetoxins)
Fibrocapsa japonica (possible brevetoxins)
Heterosigma akashiwo (possible brevetoxins)
Pseudo-nitzschia cuspidata (possible domoic acid)
Pseudo-nitzschia heimii (possible domoic acid, non-toxic in New Zealand)
Pseudo-nitzschia lineola (possible domoic acid)
Pseudo-nitzschia multistriata (possible domoic acid, non-toxic in New Zealand)
Pseudo-nitzschia subfraudulenta (possible domoic acid)
Pseudo-nitzschia subpacifica (possible domoic acid)

Category C - Other potential toxin producing species worldwide that may be present in Australian waters.

Alexandrium angustitabulatum (possible saxitoxin and derivatives, identified in New Zealand waters)
Alexandrium acatenella (possible saxitoxin and derivatives)
Alexandrium cohorticula (possible saxitoxin and derivatives)
Alexandrium fraterculus (possible saxitoxin and derivatives)
Alexandrium fundyense (possible saxitoxin and derivatives)
Alexandrium lusitanicum (possible saxitoxin and derivatives)
Alexandrium tamiyavanichi (possible saxitoxin and derivatives)
Coolia monotis (produces cooliatoxin)
Dinophysis norvegica (Major DSP producer in Europe)
Gymnodinium aureolum (possible brevetoxins)
Gymnodinium impudicum (possible brevetoxins)
Gymnodinium pulchellum (possible brevetoxins)
Karenia bidigitata (*=Gymnodinium bidigitatum*) (possible brevetoxins, found in New Zealand)
Karenia mikimotoi (=Gymnodinium mikimotoi) (possible brevetoxins)
Karenia papilionacea (=Gymnodinium papilionaceum) (possible brevetoxins) (sp. in edit).
Karenia selliformis (*=Gymnodinium selliforme*) (gymnodimine, found in New Zealand) (sp. in edit).
Karlodinium micrum (=Gymnodinium galatheanum) (possible brevetoxins)
Lingulodinium polyedra (yessotoxin producer in Japan)
Nitzschia navis-varingica (domoic acid was recently confirmed for an isolate from brackish Vietnamese waters)
Ostreopsis siamensis (produces palytoxin)
Pfiesteria piscicida / shumwayae
Prorocentrum concavum (okadaic acid?, dinophysis toxins? and diol esters?)
Prorocentrum elegans (okadaic acid?, dinophysis toxins? and diol esters?)
Prorocentrum hoffmannianum (okadaic acid?, dinophysis toxins? and diol esters?)
Prorocentrum maculosum (produces prorocentrolides)
Protoceratium reticulatum (yessotoxin producer in New Zealand)

(? Indicates this toxin has not been confirmed at the time of this report as being produced by Australian strains of this species)

7.6 Risks of Introductions of Biotoxin-Producing Organisms from Overseas

Considerable research is being carried out internationally in the areas of ballast treatment, hull de-fouling methods and anti-fouling technologies, in an effort to ensure that undesirable organisms (e.g. the Chinese mitten crab and the Asian clam) are not introduced into new areas. The Centre for Research on Introduced Marine Pests (CRIMP), Hobart, is a world-leader in risk assessment for ballast water introductions and the development of surveillance and incursion response protocols for marine pests. The risk of introductions of harmful marine organisms from overseas has been discussed fully by Hallegraeff and Bolch (1992) and Hallegraeff (1998).

Australia already hosts most of the known coastal and estuarine inhabiting toxic micro-algal species, but there are some species yet to be identified in Australian waters. For example, the following toxic dinoflagellates occur in New Zealand but are not known in Australia: *Alexandrium angustitabulatum, Karenia selliformis* and *K. bidigitata.* Other potential and undesirable microalgal introductions are *Heterocapsa circularisquama*, which has caused massive mortalities of shellfish in Japanese and Korean waters, and *Pfiesteria piscicida* and *P. shumwayae,* known from the eastern U.S. seaboard, but possibly ubiquitous. *P. shumwayae*, a dinoflagellate that can kill fish and cause neurological disorders in humans, was recently identified in estuarine water samples from Tasman Bay, New Zealand, and has also been identified from NSW and Tasmanian waters. *Pfiesteria* attacks shellfish larvae (Shumway *et al.* 2000), but there is no indication to date that its water soluble toxin is passed up the food chain. Molecular based probe assays are available to detect the presence of these species and models of contingency plans are available (Rublee *et al.* 1999; Mangien 2000; Rhodes 2000).

Australia takes the risk of marine invaders seriously and both current and proposed regulatory measures will reduce the risk of entry, although total exclusion is impossible. The Australian Quarantine and Inspection Service (AQIS) (operating within the Department of Agriculture, Fisheries and Forestry – Australia) is the Commonwealth agency responsible for management of ballast water and hull-fouling issues. The Quarantine Act 1908 underpins actions to prevent border introductions, with the latest amendment being the Quarantine Amendment Act 1999. Voluntary ballast water management guidelines were introduced in 1990, and in 1991 the International Maritime Organisation also introduced such guidelines for world shipping (based on the Australian model).

From July 1, 2001, mandatory arrangements came into play. Written permission must be obtained from a quarantine officer to discharge ballast water in Australian ports or waters. Ballast water must be managed through exchanging water at sea (or equivalent on-board treatment systems). The new Australian ballast water "Decision Support System" requires details of proposed uptake and/or discharge to be provided at the last port of call or five days prior to arriving in Australia (information on internet: www.aqis.gov.au/shipping).

If HABs are collected in ballast water from the coastal waters of one state and transported to another where shellfish are being harvested, the risks of bloom activity, and therefore marine biotoxin contamination in the recipient area, will be increased. Transport of viable bodies of *Pseudo-nitzschia* "bloom" water between ports has been observed in New Zealand (Lesley Rhodes, personal observation).

There is a substantiated risk of introductions of toxic microalgae from overseas, and of translocation of bloom water between ports within Australia (Hallegraeff 1992; Rhodes 1998). In the case of

potential ballast water introductions, the current voluntary regulations, soon to be replaced by mandatory action (July 2001), should reduce this risk. However, introductions of marine invaders via hull fouling has been demonstrated overseas. This issue is being addressed through the current AQIS reviews.

7.7 Conclusions

The monitoring of toxic microalgae to provide risk assessments of biotoxins in shellfish has proven merit. It is likely that at different times all states will be at risk from HABs, and apart from some species which are restricted to either tropical or temperate waters, most of the listed microalgae will be found throughout Australian waters. Microalgae that have not yet been recorded in Australian waters are likely to be observed as monitoring increases. Monitoring can therefore also offer some predictive capability, and this is certainly the case with identifying cyst beds.

Most of the algae are easy to identify under the light microscope, but for those that are difficult to differentiate from other morphologically similar species there are convenient identification technologies available.

Monitoring must allow for such variables as “per cell” toxin production and toxin uptake by shellfish, and while all shellfish can take up biotoxins, different species present differing uptake rates. For example, the Pacific oyster (*Crassostrea gigas*), rarely exhibits as high a toxin concentration as blue mussels (*Mytilus edulis*) when harvested from the same site.

The producers of the various toxins are well known, although new bioactive compounds are being discovered. Close links between researchers, regulators and industry stakeholders will help bridge this knowledge gap.

8 TEMPORAL AND REGIONAL OCCURENCES OF HAZARDOUS LEVELS OF BIOTOXINS

8.1 Introduction

Research carried out in Australia over the last two decades, much by the University of Tasmania and CSIRO, Hobart, make it clear that the majority of toxic microalgae known worldwide are present in Australia, although there is a tropical and a temperate component to the phytoplankton. The global spread has possibly been enhanced by ballast water translocations.

However, presence does not translate immediately to toxicity in shellfish, and conversely the lack of monitoring in some states means that where there is no data linking bloom and toxin events, there can be no confidence that blooms have not occurred.

There is a growing body of evidence that HABs have increased steadily over the last few decades, and that with the rapid increase in aquaculture over wild harvest of shellfish, that the economic costs have also increased (Anderson *et al.* 2000; CENR 2000). This report reflects the sparse monitoring data available for some states, and its use in predicting future occurrences is therefore limited to those states with reasonable historical data.

8.2 Microalgae Posing a Biotoxin Threat to Shellfish in Australian Waters: Regional and Seasonal Risk

8.2.1 New South Wales

(i) DSP-toxin producing species:

Dinophysis acuminata and *D. tripos*, which produce DSP toxins, have been suspected of being responsible for illnesses resulting from the consumption of contaminated pipis at Ballina, late 1997, and Anna Bay/Stockton Beach, Newcastle, in early 1998. The *Dinophysis* species were abundant in pipi stomachs at that time. There have been harvesting suspensions since that time due to high numbers of *Dinophysis* species in the water, but no further illnesses. A recall of pipis from South Ballina Beach occurred in 1999 due to contamination with pectenotoxin analogues.

Areas where blooms have occurred: *D. acuminata* and *D. caudata* blooms have been responsible for several ocean beach closures for pipi harvest since 1998. The 1997/8 events at Ballina and Newcastle were probably due to unrecorded *D. acuminata/D. tripos* blooms, as evidenced by the pipi gut contents at that time.

Seasonal risk: October to March. (This might need to be extended once more data is available.)

(ii) ASP-toxin producing species:

The diatom genus *Pseudo-nitzschia* is common throughout Australian waters. *Pseudo-nitzschia australis* and *P. multiseries* are the most toxic species in the group, and both have been recorded in NSW waters.

Areas where blooms have occurred: *Pseudo-nitzschia multiseries* was recorded in Berowra Creek in 1993 and 1995 (Gustaaf Hallegraeff, University of Tasmania, Australia, pers. comm.). Blooms of *P. pseudodelicatissima* have also been recorded in Berowra Creek, October 1998, with no resultant domoic acid in shellfish, but it should be noted that overseas strains of *P. pseudodelicatissima* have been shown to produce low levels of domoic acid per cell (for example, New Zealand, United States).

A mixed bloom of *P. pseudodelicatissima* and *P. pungens* closed Wagonga Inlet in October 1999, but no domoic acid was detected in oysters. Again it should be noted that *P. pungens* can produce high levels of domoic acid per cell in overseas strains.

Seasonal risk: Year round.

(iii) PSP-toxin producing species:

Alexandrium catenella:

Areas where blooms have occurred: *Alexandrium catenella* can be present in extremely low numbers and still cause PSP contamination of shellfish, and therefore its presence, rather than just bloom events, is of concern. It was recorded in Sydney Harbour, November 1993, when it was associated with 3 mg STX equiv./kg in wildstock oysters and low levels in prawns (NB no commercial farming or harvesting occurs in Sydney Harbour) . *A. catenella* was also recorded in Shoalhaven River in 1993. Resting cysts of *Alexandrium* spp. have been found in the sediments of Botany Bay (1993) and in oyster guts from Port Stephens (1991), although toxicity is unknown.

Seasonal risk: Most likely early summer through to autumn.

(iv) NSP-toxin producing species:

The potential brevetoxin producers, *Karlodinium micrum, Heterosigma akashiwo* and *Chattonella antiqua/ marina* have all bloomed in NSW waters, although toxin concentrations in all these species are probably low.

Areas where blooms have occurred: *Chattonella* sp. bloomed in Sydney Harbour and Parramatta River in the summer (November to January) of 1996/7. Blooms of *H. akashiwo* and *K. micrum* have been recorded at Berowra Creek, close to oyster harvesting and prawn trawling areas in the Hawkesbury River.

Seasonal risk: Late spring through summer (*Chattonella* has been recorded in April in SA).

It is worth noting that seawater samples containing the marine blue-green alga *Trichodesmium* sp. caused a toxic effect on mice, leading to closures of oyster harvesting at Bateman's Bay during Easter 1998.

8.2.2 Northern Territories

Problem species:

No phytoplankton events linked to toxicity in shellfish have been reported, although toxic species have been detected in the region.

No biotoxin testing of bivalve shellfish has been carried out to date and Fisheries Division, Department of Primary Industries and Fisheries, have no records of shellfish poisonings in the state.

Significantly, *Pyrodinium bahamense* var. *compressum*, which causes major PSP events in neighbouring tropical Indo-West Pacific, has been detected as resting cysts in the Port of Darwin.

Pseudo-nitzschia (ASP) and *Dinophysis* (DSP) are also present.

8.2.3 Queensland

No phytoplankton events linked to toxicity in shellfish have been recorded to date.

No routine phytoplankton monitoring is carried out, although some studies have been carried out by the Queensland EPA and the University of Queensland. The causative microalgae of all the potential biotoxins have been recorded at different times in Queensland waters, including such sub-tropical genera as *Ostreopsis* (palytoxin-like compounds such as ostreocin) and *Coolia* (cooliatoxin).

Biotoxins (PSP and ASP) are tested for under the Queensland Shellfish Water Assurance Monitoring Program, which commenced in 1993. The program is focussed on Sydney Rock oysters in Moreton Bay, and no PSP toxins or domoic acid contamination have been reported over that time. The sampling is limited, however, and as the causative organisms are present in the area it is conceivable that toxic events could have occurred and been missed due to depuration.

8.2.4 South Australia

(i) PSP-toxin producing species:

(A) *Alexandrium minutum, Alexandrium catenella*:

Areas where blooms have occurred: *Alexandrium minutum* has been regularly recorded in Port River and West Lakes since 1986 and there is a risk that blood cockles could become contaminated. The cockles are harvested recreationally and commercially (sold directly on the wharf) from just outside the Port River. *Alexandrium catenella* is also found in the Port River, and an unidentified *Alexandrium* species, linked to PSP contamination of razorfish (below regulatory closure levels) but not oysters, has been recorded along the southern York Peninsula.

Seasonal risk: Year round.

Alexandrium minutum occurs in spring and autumn when temperatures are 16°C or greater; blooms are favoured by salinities of 35 ppt and high nutrient conditions (Canon 1996). *Alexandrium catenella* bloomed April 1998; *Alexandrium* sp. was detected December-January 1998/99; January 2000.

(B) *Gymnodinium catenatum*:

Areas where blooms have occurred: No events have been linked to this microalga in South Australia, but high concentrations of cysts have been recorded in Spencer Gulf sediments.

Seasonal risk: In Tasmania the risk season has been identified as post rain in autumn and spring, when temperatures are at or above 12°C.

(ii) DSP-toxin producing species:

Dinophysis spp.:

Areas where blooms have occurred: Widespread. There have been links between the presence of *Dinophysis acuminata* and *D. caudata* blooms and pectenotoxins in oysters and mussels; harvesting closures were made in 2000 in Streaky Bay, Coffin Bay, Port Lincoln and Nepean Bay. Mt Dutton Bay harvesting area was closed for 7 months (based on 10 µg/ 100 g flesh).

Seasonal risk: Spring and summer, with blooms commonly linked to water stratification.

(iii) NSP-producing species:

(A) *Karenia* cf *brevis* and *K. mikimotoi:*

K. breve in Florida, USA, is a highly potent brevetoxin producer. It is probable that the species present in South Australia is a morphologically similar species, *K. papilionacea*, which produces extremely low concentrations of brevetoxins. This would explain why such low NSP concentrations have been detected in water samples from Spencer Gulf, despite high cell counts.

Areas where blooms have occurred: Frequently present in southern Spencer Gulf.

Seasonal risk: Summer (December-January) highest likelihood.

(B) *Chattonella marina/antiqua, Heterosigma* sp.: potentially produces brevetoxin-like compounds:

Areas where blooms have occurred: *Chattonella* at Port Lincoln. *Heterosigma* in the Port River.

Seasonal risk: Spring through to autumn (*Chattonella* bloomed Port Lincoln April 1996; Hallegraeff *et al*. 1997).

8.2.5 Tasmania

(i) PSP-toxin producing species:

(A) *Gymnodinium catenatum* is the key species of concern. It was first detected in Tasmania in 1980, possibly following introduction via ballast water.

Areas where blooms have occurred: South-eastern Tasmania, with high risk at Huon Estuary, Port Esperance, D'Entrecasteaux Channel and Port Arthur, and medium risk at Birches Bay, Fleurtys Point, Long Bay Reef and Hastings Bay (refer to Brown and Turnbull 2000).

Seasonal risk: High-risk periods are after rainfall in autumn and spring.

J	F	M	A	M	J	J	A	S	O	N	D
⮜- until <12^{0}C							⮜- when >12^{0}C				

(B) *Alexandrium catenella*

Areas where blooms have occurred: South-eastern Tasmania: Triabunna.

Seasonal risk: Most likely early summer through to autumn.

(ii) DSP-toxin producing species:

Several *Dinophysis* species and *Prorocentrum lima*. Dense blooms have occurred without toxin production.

Areas where blooms have occurred: Southeastern Tasmania: Triabunna, Derwent River.

Seasonal risk: *Dinophysis* blooms commonly occur from October to February (Derwent River).

(iii) ASP-toxin producing species:

Pseudo-nitzschia species. Of particular concern in Tasmania is *P. australis*, which has caused deaths of humans and marine mammals in Canada and the US.

Areas where blooms have occurred: Widely distributed.

Seasonal risk: Year round

(iv) NSP-toxin producing species:

Karenia mikimotoi, Gymnodinium aureolum: known fish killers in Tasmania with the potential to cause neurotoxic shellfish poisoning through the production of brevetoxins.

Areas where blooms have occurred: Widely distributed.

Seasonal risk: Summer.

8.2.6 Victoria

(i) PSP-toxin producing species:

(A) *Alexandrium catenella, A. tamarense*

Areas where blooms have occurred: *Alexandrium catenella* blooms have been recorded in Hobsons Bay (near the mouth of the Yarra River) and surrounding waters. PSP has been regularly detected in Port Philip Bay shellfish since 1988 (excepting 1996/7), for example in wild stock mussels. There were bloom alerts in the summers of 1998, 1992, 1994, 1995.

Low numbers of *A. tamarense* were linked to PSP in Port Philip Bay, July 1993. Cysts and motile cells of this species have been found in ports and harbours along the western Victorian coastline.

Seasonal risk: Summer is the prime bloom period for *A. catenella*. However, *A. tamarense* has been responsible for PSP in shellfish in winter, and so year round monitoring would be advisable.

(B) *Gymnodinium catenatum*:

Areas where blooms have occurred: No blooms have been recorded, but cysts and motile cells have been detected in most ports and harbours along western Victorian coastline and may have been responsible for PSP in abalone and rock lobsters.

Seasonal risk: In Tasmania high-risk periods are after rainfall in autumn and spring.

(ii) NSP-toxin producing species:

Karenia cf *brevis* (=*Gymnodinium* cf *breve*):

It is possible that the species observed in Victorian waters is *K. mikimotoi* or *K. papillionacea*. Both are found in New Zealand waters and are capable of producing brevetoxins.

Areas where blooms have occurred: *Karenia* cf. *brevis* was possibly responsible for massive fish deaths in Port Philip Bay in the 1950s, and a *Karenia* cf. *brevis* bloom in January 1994, at Tamboon Inlet, Gippsland Coast, was directly related to NSP in wild mussels.

Seasonal risk: Summer.

(iii) ASP-toxin producing species:

Pseudo-nitzschia species:

Areas where blooms have occurred: *P. pseudodelicatissima* blooms are common throughout Victorian waters, including Bass Strait, where the supply of whole live scallops to Asian markets is in the pipe-line. No toxicity has been associated with these blooms to date, but strains of *P. pseudodelicatissima* have proved toxic in other countries. Domoic acid has been detected in scallops from Victorian waters however, and the highly toxic species *P. australis* and *P. multiseries* have been reported recently. Concentrations of domoic acid of >600 ppm have been recorded in whole scallops in New Zealand as a result of *P. australis* blooms (Rhodes 1998).

Seasonal risk: Year round.

(iv) DSP-toxin producing species:

Dinophysis acuminata:

Areas where blooms have occurred: A bloom of *D. acuminata* occurred in 2000 in Port Philip Bay with associated toxicity (PTX2sa; PTX2; OA).

Seasonal risk: Spring and summer, with blooms commonly linked to water stratification.

The diatom, *Rhizosolenia chunii*, should also be noted. A massive bloom occurred in Port Philip Bay, October 1987, causing a bitter taste in shellfish, and shellfish mortalities several months after the bloom.

8.2.7 Western Australia

Problem species:

No toxic algal blooms or events have occurred in commercial areas to date, and no illnesses have been attributed to biotoxins, despite the presence of potentially toxic species in Western Australian waters during this time. However, DSP toxins in mussels have been linked to the presence of *Dinophysis acuminata* (OA and PTX), *Prorocentrum lima* (OA) and *P. mexicanum* (OA).

Also of concern is the presence of *Alexandrium minutum* (PSP), *Pseudo-nitzschia* (ASP), *Karenia mikimotoi* and *Gymnodinium aureolum* (NSP), and *Karenia selliformis*, which can produce gymnodimine.

Phytoplankton and biotoxin monitoring have been carried out in tandem since 1994, with phytoplankton monitoring being stepped up recently. The responsible agencies for administering

the Western Australian Shellfish Quality Assurance program are the Health Department Western Australia and Fisheries Western Australia, and the areas of focus have been Cockburn Sound and Oyster Harbour, where mussels and oysters are cultured. Phytoplankton monitoring data have also been supplied by the Waters and Rivers Commission in relation to public health.

8.3 Summary

Harmful algal bloom events (HABs) resulting in biotoxins in shellfish have been collated from information provided by regulators from all Australian states. The limited number of monitoring programs means predictions of HABs are difficult, although there is good information on some species for Tasmania, Victoria, New South Wales and South Australia. Major blooms have been recorded in Tasmania (*Gymnodinium catenatum*), Victoria (*Alexandrium catenella*), and South Australia (*Alexandrium minutum*). The following is a state by state summary:

New South Wales - Limited phytoplankton and biotoxin monitoring is undertaken by NSW SQAP, and some independent monitoring is carried out e.g. by the Newcastle Port Authority and Hornsby Shire Council (oysters), and the pipi harvest industry. Pectenotoxin from *Dinophysis acuminata* and *D. tripos* was suspected as being responsible for human illnesses following the eating of contaminated pipis (Ballina/Newcastle in 1997/8). *Alexandrium catenella* has been associated with low levels of PSP toxins in wild oysters and prawns in Sydney Harbour (1993).

Northern Territories - No biotoxin testing of bivalves has been carried out in NT; some research data indicates the potential for toxic algal impacts, for example *Pyrodinium bahamense* var. *compressum* (paralytic shellfish poisoning; PSP).

Queensland - No routine phytoplankton monitoring is carried out, although research studies indicate that toxic microalgae do occur in Queensland waters. No PSP or ASP, tested for under the QSWAMP since 1993, has been detected in Moreton Bay's Sydney Rock oysters, although sampling is limited.

South Australia – The PSP producing *Alexandrium* species are common in SA, particularly in Port River and West Lakes; cysts of *Gymnodinium catenatum* (also PSP) are present in high numbers in the Spencer Gulf. There are links between *Dinophysis* blooms and pectenotoxins in oysters and mussels, particularly in Streaky Bay, Coffin Bay, Port Lincoln and Nepean Bay. *Karenia* spp. which can cause neurotoxic shellfish poisoning (NSP) is common in Boston and Proper Bays, and *Chattonella marina* (which also produces NSP) has formed massive blooms at Port Lincoln.

Tasmania - Current monitoring carried out under the TSQAP, indicates that regular phytoplankton monitoring does provide an early warning system. There is emphasis now on phytoplankton monitoring backed up by flesh testing, and problems appear confined to waterways in Southeast Tasmania. There are gaps in the monitoring for other toxic species known to occur in those waters, for example, *Alexandrium* (PSP), *Pseudo-nitzschia* (ASP) and the DSP producers.

Victoria - VSQAP has been operating since 1987 and phytoplankton and biotoxin monitoring has been carried out since then in Port Philip Bay and Flinders. Perhaps as a result, all the main toxins have been recorded. Blooms of *Alexandrium* (PSP) and *Karenia* (NSP) have been of greatest concern and are likely to continue to pose problems in the future. Recent blooms of *Dinophysis acuminata* have been directly linked to pectenotoxins and okadaic acid in cultured mussels in the Bay.

Western Australia - Phytoplankton and biotoxin monitoring have been carried out in tandem since 1994 (HDWA and FWA, phytoplankton data also supplied by WRC), with no toxic algal blooms or

biotoxin events occurring in commercial areas to date. However, DSP toxins in mussels have been linked to the presence of *Dinophysis* and *Prorocentrum*, and the potentially toxic genera *Alexandrium* (PSP), *Pseudo-nitzschia* (ASP), *Karenia* and *Gymnodinium* (NSP) are common.

9 RISK ASSESSMENT

9.1 Introduction to Risk Assessment Concepts

Health is a key resource. It is essential if we are to grow and develop economically, and increase productivity. The close ties between the economy, the environment and health mean that all three need to be integrated in any decision-making process which seeks to be ecologically sustainable. Throughout the world there has been an increasing problem of marine biotoxin blooms in coastal marine waters, killing invertebrates, wild stocks and cultured fish, or making shellfish and fish toxic due to the accumulation of algal toxins which can intoxicate human consumers. By understanding and identifying risks early on, and establishing means of assessing and controlling existing hazards, public health can be improved, and the benefits associated with development can be enjoyed.

If we wait until adverse effects are detected, it may be too late to prevent human suffering. UNESCO 1996 believes that the effects of harmful algal blooms must be minimised through proper management of the environment and the resources based upon well focused harmful algal bloom monitoring programs (Andersen 1996).

The Risk Analysis model is being used by reputable agencies around the world to ascertain how best to manage environmental problems such as marine biotoxins. Organisations such as the World Health Organisation, the US Environmental Protection Agency and the National Health and Medical Research Council, Australia, have endorsed this model.

The National Health and Medical Research Council, Australia recommends that Risk Analysis should be comprised of:

Risk Assessment - What are the risks and/ or benefits?
- Who will be affected and to what extent?

Risk Communication - Has there been adequate consultation on the risks?
- Have the public concerns been taken account of?

Risk Management - Can risks be avoided or reduced?
- What are the options for treating the risks?
- Are contingency and emergency plans adequate?
- How can differing perceptions of risk be mediated?
- Can future health risks be predicted?

9.1.1 Method of Risk Assessment

The accepted protocol for undertaking a Risk Assessment is to complete four steps:

(i) Hazard Identification – assess available evidence on the presence and hazards of organisms.

(ii) Dose-response assessment – determine the effects at different doses.

(iii) Exposure assessment – estimate the magnitude, duration and frequency of human exposure to the organism of concern.

(iv) Risk characterisation – combine the information from the above three steps to estimate the risk associated with each exposure scenario considered, and to present information on uncertainties to risk managers.

Quite often when undertaking a Risk Assessment, information is unknown or unattainable to complete all four steps.

Risk Assessment is not an exact science. It estimates the probability, under certain circumstances, that an event will occur. Because of insufficient information or lack of scientific evidence, most risk assessments rely on assumptions or extrapolations. Other contributors to uncertainty include variability in exposures at different locations and times; variable individual susceptibility and response to exposure; and unknown, synergistic, long term, or delayed effects. The often-unavoidable uncertainty in risk assessment makes it important that each assessment includes a clear statement about assumptions and uncertainties. It is recommended that these be managed in the following way:

9.1.2 Dealing with Assumptions and Uncertainty

Assumptions and scientific judgements should be clearly stated.

The nature and magnitude of uncertainties must be explained.

Areas where there is lack of scientific knowledge must be clearly stated.

Risk assessment and risk management should both be addressed.

The choice of a particular risk assessment methodology over alternative methodologies must be explained.

Risk estimates must be presented to afford comparison of risks

9.2 Goal of Marine Biotoxin Risk Assessment

The first step before starting a risk analysis is to decide what the "risk" actually is. This is due to the fact that risks are perceived differently amongst a variety of persons, depending on political, economic and cultural biases.

Because of these potential differing opinions, it is also important early on to identify the stakeholders in the issue, who may influence the risk perception and any resulting Risk Management strategies. For the purposes of this project the stakeholders have been listed in the project as various government agencies and industry.

It is recommended that consideration also be given to include shellfish markets and the Australian public as stakeholders.

The scope of this project requires that "the risk to public health posed by marine biotoxins be assessed". It is understood that this assessment shall relate specifically to commercial shellfish activities and does not incorporate the risk to the recreational shellfish harvester and consumer.

The consumption of shellfish affected by marine biotoxins has the potential to directly affect public health. However, public health is also indirectly affected by economic factors (Baker and Illsley 1990). If the commercial shellfish industry is affected by marine biotoxins there are likely to be economic consequences.

Adverse economic effects will be caused by:

i) the inability to harvest safe shellfish
ii) potential loss of markets due to perceived or actual risks.

This loss of income and or employment opportunities may cause adverse health effects to the fishing industry and the general community.

This Risk Assessment will therefore consider:

1) Risk of potential illness from the consumption of shellfish affected by marine biotoxins.

2) Risk of economic losses due to failing market marine biotoxin requirements for shellfish.

9.3 Risk Assessment

The risk assessment will consider the Australian data in association with the four steps: Hazard Identification, Dose Response, Exposure Assessment and Risk Characterisation.

9.3.1 Assumptions and Uncertainties

The following assumptions and uncertainties were identified during this assessment:

1) This risk assessment only relates to Australian commercial shellfish. Recreational harvests are not considered in the scope of this risk assessment.
2) There is inadequate phytoplankton and shellfish flesh data for all states in Australia to be able to:
   (i) quantify the risks associated with marine biotoxin contamination
   (ii) predict seasonal patterns for events
   (iii) quantify differences in toxicity between shellfish species.
3) The "dose-response" levels for this risk assessment were considered to be internationally established regulatory toxin levels.
4) It is assumed that all TSP cases reported in this document were investigated and confirmed using sound epidemiological practices.
5) Internationally marine biotoxin events are unpredictable. The knowledge of the environmental and biological factors that cause events is not adequate to predict patterns and severity of TSP events – even in countries that have many years of sampling information.

9.3.2 Step 1: Hazard Identification

The hazards for the purpose of this risk assessment are the toxins that cause TSPs. The health effects of TSPs are documented in Section 1.3 of this report.

Each of these TSP groups is associated with specific species of microalgae. However, the presence of these microalgal species in the marine environment does not automatically mean the presence of the toxins in shellfish. Toxin production depends on a number of environmental and species biological factors, many of which are not well understood. It is not possible at this stage to accurately predict actual shellfish toxicity from microalgae species presence.

It is important to remember that although the toxins are produced by species of microalgae, the "hazard" is not actually the microalgae but the toxins that they produce. Therefore, although the presence of these species in a marine environment indicates potential hazard but it does not confirm

the actual hazard. However, many countries use microalgae as a sentinel indicator to warn of the likelihood of toxin (hazard) presence.

Microalgae strains associated with TSP have been isolated in Western Australia, South Australia, Tasmania, Victoria, New South Wales and the Northern Territory. Queensland, Northern Territory and New South Wales do not routinely perform phytoplankton monitoring in some or all of their commercial shellfish growing areas. However, as shown in section 6 of this report, there is the potential for adverse health effects from all TSP groups in all coastal Australian states.

What is the prevalence of marine biotoxins in Australian commercial shellfish?

Routine shellfish testing programs have not been established in all states to assess biotoxin activity. Therefore it is not possible to document a comprehensive historical database on toxin activity in commercial bivalve shellfish species. Table 17 summarises the data that was collected from the state agencies for this report.

Table 17. Summary of known shellfish toxin isolations in Australian bivalve shellfish

State	Toxin isolated	Shellfish species	Illness associated
WA	DSP	Cultured blue mussels	No
QLD	None found		
SA	PSP	Razorfish	No
	DSP	Oysters and Mussels	No
TAS	PSP	Oysters and mussel	Yes – (wild stock mussels)
NT	None found – no testing undertaken		
NSW	DSP (PTX2 & PTX2sa)	Pipis	Yes
VIC	PSP	Cultured and wild stock mussels	No
	ASP	Scallops	No
	NSP	Wildstock mussels (recreational harvest)	Yes
	DSP (PTX)	Cultured mussels	No

The actual hazard has been identified in commercial bivalve shellfish in five of the seven coastal states (71%).

9.3.3 Step 2: Dose Response

Many scientific papers have been published on the toxicological aspects of marine biotoxins. The mode of action and the severity of the effect depend on the toxin type and the individual's response. As is usual for most toxicants the individual dose response is related to age, sex, weight and general health of the consumer.

This risk assessment does not reassess this toxicological information or establish dose response levels. Internationally regulatory levels have been established and these are considered appropriate levels at which to respond to. Table 18 presents these levels.

Table 18. Regulatory (dose response) levels for marine biotoxins

TOXIN	REGULATORY LEVEL
ASP	20 ppm of domoic acid in edible part of shellfish
DSP **	Equal or greater than 20 µg/100 grams in edible part of shellfish
NSP	Equal or greater than 20 MU/100 grams in edible part of shellfish
PSP	Equal or greater than 80 µg/100 grams in edible part of shellfish.

References: USFDA Guidance Levels and EU Council Directive 91/494/EEC.
** Yessotoxins and Pectenotoxins currently are classified in the DSP group. Currently there is lack of epidemiological evidence on the human health effects from these toxins and the dose response associated with these effects. There is still international debate as to what the regulatory allowable levels should be for these yessotoxins and pectenotoxins. However, currently many countries only allow the 20 µg/100 grams for yessotoxins and pectenotoxins.

9.3.4 Step 3: Exposure Assessment

The lack of robust sampling information makes it difficult to provide quantifiable exposure data.

Commercial bivalve shellfish is harvested from all coastal Australian states. The amount of production from each state is listed in Table 19.

Table 19. Tonnes of bivalve production in states for period 1999 –2000

SPECIES	NSW	VIC	QLD	WA	SA	TAS	NT	CW
Scallops	-	346	7398	2756	-	423	2	22
Oysters	5584	-	143	-	2494	4748	-	-
Mussels	50	957	-	683	81	-	-	-
Cockles	42	-	-	-	329	-	-	-
Pipis	481	-	-	-	-	-	-	-

Reference: Fisheries Economics Section, ABARE

This shellfish is sold within states, between states and some is exported.

The Australian Seafood Industry Council provided the following information on the export dollars earned by bivalve shellfish for the 1996-1997 period.

Scallops A$36,570,000
Oysters A$296,000.

The group of the population at risk are those who eat Australian commercially sold shellfish - either in Australia or overseas.

Bivalve species differ markedly in their ability to uptake and eliminate toxins. Mussels uptake toxins quite quickly and are therefore often used as a sentinel species. Oysters accumulate toxins, but in most cases tend to exhibit lower toxicity than mussels irrespective of the species of oyster or type of toxin. Some shellfish species are more susceptible to specific toxin groups e.g. scallops are more affected by ASP. Again due to the paucity of specific Australian data it is not possible to quantify the risks associated with the different shellfish species and regions. However, blue mussels and scallops are considered as the higher-risk species in Australia for potential TSP events (see section 6).

It is likely that there are seasonal differences in toxin prevalence, but due to lack of Australian data it is again not possible to quantify this.

No information was obtained for this risk assessment on the Australian shellfish consumption patterns. Therefore it is not possible to give demographic details on the typical shellfish consumer e.g. age, sex, ethnicity nor on specific amounts of shellfish consumed within a nominated time period.

Details were obtained from each state on documented TSP illness events. The epidemiological investigations and TSP confirmation were not verified as part of this Risk Assessment. An assumption was made that they were positive TSP cases. It is always extremely important that if public policy decisions are made on information, that this information be sound. Therefore, it may be appropriate to verify the epidemiological investigation and confirmatory steps that were undertaken for the TSP cases documented in this report.

A summary of the known TSP cases are depicted in Table 20.

Table 20. Number, type and year of TSP cases in Australia associated with shellfish species

State	No. Of cases & year	Tsp type	Shellfish species
Tasmania	2 (1993)	PSP	Mussels – recreational harvest.
New South Wales	50 (1997)	DSP (PTX2) & PTX2sa	Pipis – commercial
	20 (1998)		Pipis - recreational
Victoria	? * (1994)	NSP	Mussels – recreational.

* Exact numbers of cases not documented. It is probable that these cases were not epidemiologically investigated and confirmed.

9.3.5 Step 4: Risk Characterisation

It is not possible to give conclusive quantitative data on the marine biotoxin risks associated with the consumption of Australian shellfish. To undertake a quantitative analysis would require specific information on the toxin levels in all commercial shellfish species throughout different environmental conditions e.g. different seasons. An adequate database for Australian commercial shellfish is not available.

The conditions that cause microalgae to produce toxins are not well understood; therefore it is not possible to predict when and where the toxin events will occur. It is likely that different environmental factors affect the severity and frequency of events. However, these factors may not be simply related to annual seasonal climate events, but may be related to longer-term climate cycles.

Overseas studies have shown different species of shellfish uptake and eliminate toxins at different rates (Shumway 2000). Mussels are known to be particularly fast in accumulating toxins and are often used as the sentinel species. However, there is little comparative data available to assess the various Australian commercial species. Rhodes and Hallegraeff (section 6 of this report) comment that blue mussels and scallops are considered to be the Australian commercial shellfish most likely to be involved in future TSP events.

To date there has been few identified illnesses due to TSP outbreaks associated with Australian commercial shellfish and a total of 52 cases have been reported. 50 of these cases were in one outbreak associated with pipis in New South Wales.

There have also been TSP cases associated with recreational harvests – an unknown number of NSP cases were reported in Victoria associated with wild harvest mussels. A DSP outbreak of more than 20 persons was reported in New South Wales associated with pipis – again this was associated with recreational harvests in the Anna Bay/Stockton Beach area.

Incidence rates have been calculated as follows:

1) For the total number of TSP cases (commercial and recreational), assuming 10 cases in NSP event in Victoria. Therefore assumed total 82 cases. The population of Australia as 10th May, 2001 (Ref. Australian Bureau of Statistic) is 19,343,648. Therefore the total combined incidence rate for Australia is 4.2 cases per 1,000,000 population.

2) For the total number of TSP cases associated with commercial product (n =50), the incidence rate is 2.5 cases per 1,000,000 population.

3) The incidence rate within New South Wales (population 6,411,000) associated with commercial product (50 cases with pipis) is 7.7 cases per 1,000,000 population.

These human TSP incidents have occurred in the period 1993 –1998.

Although it is not possible to provide specific quantitative data for this report, an attempt has been made to rank the risk factors associated with TSP for each state (Table 21).

Table 21. List of risk factors associated with TSP events

	WA	SA	VIC	TAS	NSW	QLS	NT
HAB likely to be present	+	+	+	+	+	+	+
Commercial shellfish industry	+	+	+	+	+	+	+
At risk** species commercially harvested (mussels and scallops)	+	+	+	+	+	+	+
Toxin presence isolated in shellfish	+	+	+	+	+	-	-
TSP cases associated with state	-	-	+	+	+	-	-

** At risk species as considered in section 6 of this report

All states have environments highly likely to support HAB growth.

All coastal states have a commercial bivalve shellfish industry – though the amounts and varieties of shellfish differ significantly e.g. Northern Territory only has a minimal scallop industry.

The risk of a marine biotoxin event occurring, which will adversely impact public health and/or market access cannot be accurately quantified. However, if the states are ranked according to the marine biotoxin risk factors depicted in Table 21 then Victoria, Tasmania and New South Wales potentially, at present, have the highest risk of a TSP event affecting shellfish consumers.

Risk management is acknowledged to mitigate the adverse effects from actual risk events. Internationally the factors considered appropriate Risk Management for marine biotoxins are:

1) Minimum marine biotoxin monitoring requirements.
2) Need for contingency management plans, which outline how biotoxin events will be managed.

Table 22 summarises the status of Australian states for marine biotoxin management, and shows that the states deemed to have a “high risk” when using the ranking system (Victoria, New South Wales and Tasmania) also do not have comprehensive marine biotoxin management systems in all their commercial areas. This status increases their potential to suffer adverse effects from a TSP event. It is very likely a TSP event would not be managed and mitigated in a timely manner.

Table 22. Summary of marine biotoxin management status in Australia

	WA	SA	VIC	TAS	NSW	QLS	NT
Phytoplankton monitoring program	3**	3	3**	3**	7**	7	7
Flesh testing to verify toxin levels	3	3	3**	3	7***	7	7
Marine Biotoxin Management Plan	3*	3	3	3*	7***	7	7

* Plan needs amendment to ensure all aspects are adequately addressed.
** Phytoplankton monitoring not undertaken in all state commercial harvest areas.
*** Pipi harvests have marine biotoxin management in NSW. A Biotoxin Contingency/Management Plan is in place for 30 estuaries in NSW, with monitoring implemented in some areas.

The lack of compliance with internationally recognised marine biotoxin management systems has the potential to adversely affect market access - intrastate, interstate and internationally.

9.4 Who is at risk from TSP events in Australia?

Consumers of Australian bivalve shellfish are potentially at risk from adverse public health effects of TSP episodes.

Industry is potentially at risk from the adverse effects of a marine biotoxin event and from failing the regulatory market access requirements.

These risks cannot be quantified. Marine biotoxin events are recognised as being "unpredictable" in their occurrence and severity. This unpredictability occurs even in countries with historical databases based on regular monitoring of phytoplankton and toxin assays of shellfish flesh.

Australia has very little historical information on conditions in which microalgae and toxin production occur. This lack of information means that there is no ability to define risk factors in a quantifiable way. This lack of information possibly accentuates the risk of a TSP event not effectively being monitored or managed.

9.5 Conclusions

(i) There is currently lack of adequate scientific data to be able to quantify the Australian marine biotoxin risks associated with commercial shellfish.

(ii) There is little understanding on the seasonal and species prevalence of marine biotoxins within Australian commercial bivalve species.

(iii) Most of the Australian states that harvest commercial bivalve shellfish do not comply with the international requirements for marine biotoxin management – sentinel monitoring and contingency management plans.

(iv) There have been TSP cases associated with commercial bivalve shellfish in Australia. The incidence rate is low. However, it is very likely that further TSP events will occur.

(v) Using a simple ranking system it is likely that Victoria, Tasmania and New South Wales have a higher risk of a TSP event.

(vi) Internationally marine biotoxin events are unpredictable – even in areas with an understanding of environmental and biological factors that cause such events.

(vii) At present there is a lack of information of the Australian environmental and biological conditions that may cause TSP events. This lack of information possibly accentuates the risk of a TSP event not being effectively monitored or managed.

(viii) There is a risk that there will be direct adverse public health effects caused by TSP events associated with Australian commercial shellfish.

(ix) There are likely adverse economic effects to the shellfish industry from TSP events. These economic effects will indirectly affect public health.

(x) There are potential market access failures due to the lack of regulatory marine biotoxin management systems effectively operating in all commercial harvest areas. These market access failures may adversely affect the economics of the shellfish industry.

10 DATA TO UNDERPIN FOOD SAFETY CONTROL AND REGULATORY MECHANISMS

10.1 Introduction

The potential public health effects from marine biotoxins are recognised by public health and food regulatory agencies throughout the world. Most countries have regulatory requirements stating:

1) Minimum marine biotoxin monitoring requirements.
2) Need for contingency management plans, which outline how biotoxin events will be managed.
3) Maximum permissible toxin levels allowed in shellfish for sale.

10.2 US NSSP and EU

Section 2 of this report outlines a review of the international models for marine biotoxin management. The shellfish management programs as required by the US National Shellfish Sanitation Program and the European Union both depict the regulatory framework required by most countries.

10.2.1 US National Shellfish Sanitation Program

1) This requires the Authority* to develop and adopt a marine biotoxin contingency plan for all shellfish growing areas. The purpose of this plan is to outline how marine biotoxin events will be managed.
2) In those areas where marine biotoxins are likely to occur in shellfish, representative samples shall be collected during all harvest periods. Samples shall be collected from indicator stations at intervals determined by the Authority, and assayed for the presence of toxins.
3) The regulatory levels for toxins are:
    i) PSP equals or exceeds 80 micrograms per 100 grams of edible portion of raw shellfish.
    ii) Any NSP found in shellfish meats; or
    iii) The cell counts for *Gymnodinium breve* organisms in the water column exceed 5,000 per litre; or
    iv) For Domoic Acid (ASP), the toxin concentration shall not be equal to or exceed 20 ppm in the edible portion of raw shellfish.

* Authority is defined as the State or local shellfish control authority, or designated agents, which are responsible for the enforcement of this Model Ordinance.

10.2.2 European Union 91/492/EEC

The European Union has the following requirements for placing live molluscs on the market.

1) A competent authority must have a system to verify that the EU requirements are complied with.
2) There shall be periodic monitoring to check the possible presence of toxin producing plankton in the waters and biotoxins in the shellfish.
3) The regulatory levels for toxins are:
    i) PSP content in edible portions of shellfish must not exceed 80 micrograms per 100 grams of mollusc shellfish.
    ii) the customary biological testing methods must not give a positive result to the presence of DSP in the edible parts of molluscs. The EC expert DSP working group

has drafted new legislation (SANCO/2227/2001 Rev 3) which sets total DSP (OA, DTXs and PTXs) at 16 µg/100 g; YTXs at 100 µg YTX equiv./100 g; and AZA at 16 µg AZA equiv./100 g. The detection methods have also been reviewed (see page iv for details).

iii) The total Amnesic Shellfish Poison content in the edible parts of the molluscs shall not exceed 20 micrograms of domoic acid per gram using HPLC method.

If Australia wishes to export shellfish to an overseas market, there would be a requirement to comply with one or both of the above standards.

10.3 Australian Shellfish Quality Assurance Program (ASQAP)

This is a reference document for Australian Federal and State government agencies involved in the implementation of the program for all commercially harvested bivalve molluscs from Australian waters. It is not mandatory. ASQAP recommends a sampling program that acts as an early warning system that triggers shellfish sampling and a management contingency control strategy.

10.4 Australia and New Zealand Food Standards

The Australian New Zealand Food Authority (ANZFA) develops standards and associated draft codes of practice and guidelines. ANZFA also has a role in co-ordinating monitoring and surveillance activities in relation to food, and in developing food education initiatives to increase public awareness. Currently the Australian Food Code lists the following standards for bivalve molluscs.

The edible portion of bivalve molluscs

i) Must not contain a level of PSP greater than 0.8 mg/kg when determined by the method of the A.O.A.C., 15th Edition (1990), Section 959.08:

ii) Must not contain a level of domoic acid greater then 20 mg/kg when determined by the A.O.A.C., 15th Edition (1990), 2nd Supplement (1991), Section 991.26.

The New Zealand standards list regulatory levels for four toxin groups. Therefore ANZFA have recommended that the Joint Australia New Zealand Food Standards Code incorporate standards for all four toxins (Table 23).

Table 23. Proposed regulatory toxin levels for joint Australia New Zealand Food Standards Code

TOXIN	REGULATORY LEVEL
ASP	20 ppm of domoic acid in edible part of shellfish
DSP	Equal or greater than 20 µg/100 grams in edible part of shellfish
NSP	Equal or greater than 20 MU/100 grams in edible part of shellfish
PSP	Equal or greater than 80 µg/100 grams in edible part of shellfish.

10.5 Compliance Status in Australian States

The following is a summary of the marine biotoxin monitoring and management information (Table 24) as presented in section 4 in this report.

Western Australia

Has a phytoplankton-monitoring program, which triggers flesh monitoring. However, this program does not currently cover all commercial harvesting sites.

There are some elements missing in the Marine Biotoxin Contingency Plan.

South Australia
Has a marine biotoxin-monitoring program in all commercial shellfish areas and a complying contingency plan.

Tasmania
Has a marine biotoxin monitoring program but this does not cover all the commercial wild harvest areas.

The contingency plan needs minor amendments to ensure compliance.

Northern Territories
No plan and no monitoring undertaken.

New South Wales
Have a Marine Algal Biotoxin Contingency/Management Plan covering 30 estuaries. Routine phytoplankton monitoring is in place in several areas with plans to instigate monitoring in other areas in the near future. There are also Biotoxin management plans for commercial harvesting of pipis. Routine monitoring is not undertaken in all commercial mussel and oyster areas.

Victoria
Undertakes a regular phytoplankton monitoring and flesh testing program in some commercial mussel and scallop harvest areas. However, this monitoring does not cover all commercial sites.

Table 24. Summary of marine biotoxin management status in Australia

	WA	SA	VIC	Tas	NSW	QLS	NT
Phytoplankton monitoring program	3**	3	3**	3**	7**	7	7
Flesh testing to verify toxin levels	3	3	3**	3	7***	7	7
Marine Biotoxin Management Plan	3*	3	3	3*	7***	7	7

* Plan needs amendment to ensure all aspects are adequately addressed.
** Phytoplankton monitoring not undertaken in all state commercial harvest areas.
*** Pipi harvests have marine biotoxin management in NSW. A Biotoxin Contingency/Management Plan is in place for 30 estuaries in NSW, with monitoring implemented in some areas.

10.6 Discussion

There are international marine biotoxin management regulatory requirements for commercial bivalve shellfish. Both Australia and New Zealand have similar standards.
These requirements can be summarised as:

1) Marine biotoxin monitoring and management requirements.
2) Maximum permissible toxin levels allowed in shellfish.

Although shellfish is commercially harvested for human consumption from all coastal Australian states, not all states comply with the minimum requirements.

To ensure compliance states are required to have a sentinel program to monitor for biotoxin activity in all commercial harvest areas. This program should monitor for all four TSP groups.

As an adjunct to the monitoring program each state should have a documented marine biotoxin management plan that outlines the administrative procedures and resources necessary to:

i) Initiate an emergency shellfish sampling and assay program
ii) Close harvesting and embargo shellfish;
iii) Prevent harvesting of contaminated species;
iv) Provide for product recall;
v) Disseminate information on the occurrences of toxic algal blooms.

Currently only South Australia fully complies with all the regulatory requirements for marine biotoxin monitoring and management.

Although the other states may undertake some sampling, the sentinel program does not cover all the commercial bivalve harvest areas in the state. These states are not in compliance with the full contingency plan requirements either.

This status of non-compliance is a potential problem because of:
1) Potentially adverse public health outcomes due to lack of monitoring and mismanagement of actual TSP events.
2) Non-compliance with overseas regulatory requirements.
3) Inability to verify compliance with the allowable toxin levels in the Australia New Zealand Food Standards Code.
4) Lack of compliance with the recommended practices of the Australian Shellfish Quality Assurance Program.

10.7 Conclusions

To comply with the Australia and New Zealand Food Standards all states need to show evidence that they have a sentinel-monitoring program in place where marine biotoxins are likely to occur.

In conjunction with this monitoring program there is a need for states to have a documented contingency plan.

Currently South Australia complies with the regulatory requirements for marine biotoxin management. However, there are vital elements missing from the monitoring and management requirements for all other states that have commercial bivalve shellfish harvests.

This compliance status is of concern due to:
1) Lack of ability to show compliance with international and Australasian marine biotoxin and food safety legislation.
2) Potential adverse public health outcomes due to lack of monitoring and mismanagement of actual TSP events.

11 COMMUNICATION

There needs to be open communication networks between all parties involved in a monitoring program and all parties should be clear on their role. A model of the New Zealand communication network for the monitoring program is shown in Figure 6. Even though the industry and recreational programs are funded separately, they overlap, because the Public Health Officers, who are responsible for the recreational gathering program, are also responsible for the commercial program in their areas. Industry plays a big role in the monitoring, as they are responsible for their own sampling. There is also data sharing arrangements between all parties, which promote programs that work well for all concerned. Countries like Denmark operate similar communication networks.

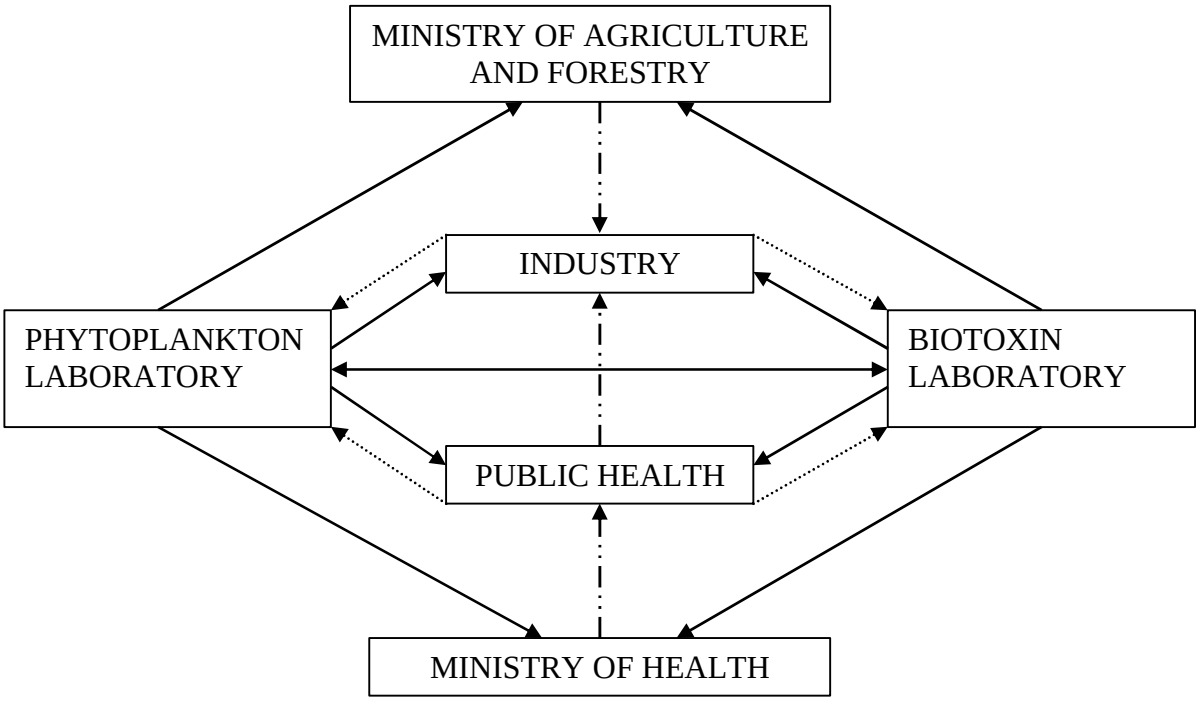


Figure 6. A simplistic model of the communication network for marine biotoxin surveillance in New Zealand.
Key: ·····▸ = samples; ——▸ = results; — · — · ▸ = actions.

A Natural Toxins working group created by SCARM (resolution 50, meeting number 16, August 2000) recommended the creation of AusToxNet to provide a mechanism for communication, consultation and coordination among industry sectors and government agencies on all matters related to the management of natural toxins affecting agricultural or fisheries products. The membership comprises:
-a representative of each major food-producing industry sector including the red meat industries, or livestock industries, grains, stockfeed, horticulture, fisheries and aquaculture industries.
-a representative of the rural industries research and development sector
-a representative of each State/Territory
-a representative of SCFA, and Commonwealth agencies with a key interest in natural toxin management such as AQIS, ANZFA, CSIRO, AGAL
-additional representatives could be co-opted to deal with particular natural toxin situations

AusToxNet will report as required to SCARM and to the SCFA. Member organisations will participate at their own cost.

The ownership and custody of data generated by phytoplankton and marine biotoxin monitoring programs is a sensitive issue. In a domestic sense, public knowledge on significant marine biotoxin problems in one area could mean an immediate market advantage for unaffected areas. Internationally, significant Australian marine biotoxin problems ought to be carefully managed not to damage the reputation of our seafood industry as a whole. While commercial market interests thus may seek to suppress public knowledge on marine biotoxin events, the occasional need for public health warnings seeks to achieve the opposite effect. Similarly, the AQIS operated decision support system covering ship ballast water translocation seeks to make it compulsory for port authorities to declare the occurrence of harmful algal blooms (analogous to cholera outbreaks being a compulsory communicable disease under WHO regulations). Important advantages may result from incorporating phytoplankton and marine biotoxin data in environmental data sets. It is recommended to store such data on a secure AFFA controlled website, with different levels of access carefully managed via passwords. The protection of seafood market interests could be partly solved by providing the seafood industry with a 1-3 month's embargo on biotoxin data, and separating phytoplankton data from (the more sensitive) shellfish biotoxin data.

A further source of expertise that could be tapped into is the Australian Research Network for Algal Toxins (ARNAT). This is a volunteer network of researchers within Australia linked by their common interest in all aspect of both marine and freshwater toxic algae including cyanobacteria and their toxins. The ARNAT web page can be accessed at www.aims.gov.au/arnat, and over time will develop a Directory of Research Activities, Directory of Experts, Directory of Blooms and Directory of Facilities and Analyses. ARNAT can act only as an advisory body but has no formal jurisdiction.

12 FUNDING

A number of important research questions (e.g. the unknown human oral potency of pectenotoxins; the concern for neurotoxic compounds produced by the tropical cyanobacterium *Trichodesmium*; the unknown potential for *Ostreopsis* and raphidophyte toxins to accumulate in shellfish) should be pursued by individual scientists/industry sectors through funding applications to FRDC (Fisheries Research Development Corporation) or Australia Research Council (ARC).

However, the significant costs of routine monitoring programs cannot be covered by such granting agencies, and need to be funded by a levy on the seafood production industries taking into account the risk profile of the geographic locality and fisheries products concerned. In New Zealand, the shellfish industry pays for all of the monitoring undertaken in commercial areas on a user pays system, whereas the Ministry of Health monitors the non-commercial areas. The Ministry of Health (MoH) and industry have a data sharing agreement that allows the MoH to use commercial data to assist in dealing with marine biotoxin problems in nearby recreational areas.

The sharing of phytoplankton and marine biotoxin data between the Seafood Industry and Environmental Protection Agencies, Water and Sewage authorities (eutrophication), Port Authorities (introduced pests), Aboriginal Commissions (Northern Territory) would enhance the coverage of the data and reduce the cost for the shellfish industry. For example, the $11M Port Phillip Bay environmental study, funded by Melbourne Water to define the impact of sewage nutrients, heavily relied on phytoplankton data collected by the Victorian Shellfish Quality Assurance Program. Ideally there needs to be a national database that contains all phytoplankton, biotoxin and environmental information from each state in a central system, which is capable of reporting when required or can be put towards a bloom prediction model.

Only after Federal and State Governments have made a clear regulatory commitment to maintaining and policing biotoxin standards, can commercial interests afford to invest in building up Australia's much needed analytical expertise in toxin chemistry and phytoplankton monitoring. A system of laboratory accreditation is urgently needed, as the limited Australian expertise available to date is of highly variable quality.

13 CONCLUSION

Given that Australia has had relatively few biotoxin incidences, it would be very easy for people to become complacent about the actual risk of biotoxin contamination in shellfish. New Zealand was in a very similar situation prior to 1993, until a nationwide closure was necessary following 180 illnesses fitting the case definition for NSP. This highlights the need for the shellfish industry and regulators to be pro-active and educated about the issue of biotoxins, and be prepared for events rather than reactionary.

Currently there appears to be very little data sharing between states, and even within states. As there is little state or federal funding available to finance marine biotoxin programs, any testing that is carried out by industry tends to be kept within the industries knowledge. Given that relatively little is known about marine biotoxin occurrences in Australia, it would appear to be important that any information that is available is shared. Education of everyone involved in the area is a key component of a successful marine biotoxin program.

A mechanism needs to be put in place within each state to regularly get all concerned parties together to discuss issues, share information and generally promote openness between parties. One way of doing this is in the form of workshops to discuss specific items. Guest speakers could be invited to discuss issues such as regulations, management plans, phytoplankton species, biotoxins, research ideas, research that is currently being undertaken, funding issues amongst a variety of other topics. By inviting industry, regulators, health officers, fisheries officers, laboratory personnel etc the issues can be discussed with input from all interested parties. New Zealand has six monthly Marine Biotoxin Workshops that work on this format. It is hosted by MAF who provides the venue, lunch and morning and afternoon teas and is attended by researchers, health protection officers, industry personnel, laboratory staff, regulators and any other interested people. Various people give short presentations, followed by a discussion time. This is an excellent means of ensuring everyone involved in the marine biotoxin scene is up to date with the latest research, it promotes openness and helps ensure that research isn't duplicated, so funding can be efficiently used for a range of research topics. This openness has also promoted the funding of research projects as it shows the Foundation for Research, Science and Technology (FRST) that the scientific community has the support, and knowledge, of the industry they are working for.

For a national strategy to work there needs to be national sharing of data and knowledge. And within each state, the industry and appropriate government authorities need to work closely together to ensure they are collecting meaningful phytoplankton data, biotoxin data and environmental information. This should be collected with the aim of the data being used to create a predictive model. A national database that is maintained and kept up to date that contains all of the information from each state in a central system, and which is capable of reporting that data when required should be investigated. It is important that data collected is used in an ongoing manner to ensure that models can be developed that highlight the areas of greatest risk, yet don't exclude those areas that haven't had problems.

The data collection should not be restricted to only phytoplankton cell counts and biotoxin levels, it should include a variety of environmental parameters also. At a minimum, temperature and salinity should also be measured. By taking vertical profiles of these parameters, water column stratification can be investigated, as this is a commonly held belief that blooms follow a period of heavy rainfall and run-off, which is subsequently followed by a period of intense sunlight. It is also important to include measurements of nutrients (in particular macro-nutrients). Smayda (1995) suggests measurement of inorganic nitrogen and phosphorous, and oxygen as a minimum.

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General information on HAB: http://ioc.unesco.org/hab/data32.htm

Information on monitoring for *Pfiesteria* in Maryland, U.S.*:* http://www.dnr.state.md.us/pfiesteria/

Abstracts from the symposium on Harmful Marine Algae in the U.S., Woods Hole 2000: http://www.redtide.whoi.edu/hab/symposium/Abstracts_master.doc-1.pdf

NATA website - http://www.nata.asn.au/

http://www.asic.org.au;

http://www.anzfa.gov.au;

http://www.seafood.co.nz

www.aims.gov.au/arnat

Appendix 1 State Program Managers Questionnaire

EXISTING STATE BIOTOXIN PROGRAMS

Questionnaire/Information Request for State Shellfish QA Program Leaders to assist development of Australian National Biotoxin Strategy

Shellfish Resources

1. What edible bivalve shellfish exist in your State? Give common and scientific names and include scallop species if whole body or 'roe-on' meats are eaten.

2. What is the distribution of each of the above species in State coastal marine or estuarine waters? Include distribution maps if available.

3. What species are currently harvested *commercially* and what species are currently cultured *commercially* in your State?

4. Are there plans to harvest or culture other shellfish that are not currently sold commercially?

5. For each commercial *aquaculture* species (oysters, mussels, etc.) give the location of all aquaculture zones in each estuary/embayment and coastal section and the number and average size of the leases in each aquaculture zone. Include distribution maps if available.

6. For each commercial *wildstock* species (scallops, pipis, cockles, clams, etc.) provide a list of estuaries/embayments and coastal sections where shellfish can be harvested commercially. Include distribution maps if available.

7. What shellfish species can be harvested *recreationally* by the public for human consumption and are 'open areas' the same as those indicated above for the commercial harvesting of the same species? Describe differences between recreational and commercial areas if they occur.

8. Is the commercial or recreational harvesting of wildstock resources of any shellfish species for human consumption prohibited? If so, what is the reason for the restriction? Environmental or shellfish protection reasons OR health protection reasons including possibility of biotoxin poisoning?

9. If the commercial or recreational harvesting of a wildstock shellfish species is prohibited for human consumption purposes, can the relevant species be harvested by industry and/or the public solely for bait?

Biotoxin Management

10. Is your agency the sole or lead agency with responsibility for biotoxin management/biotoxin monitoring in your State? If there is more than one agency involved list the other agencies and their responsibilities, and provide contact names, addresses, and telephone/fax/e-mail details for these agencies.

11. Are you personally the officer responsible for biotoxin management in your State? If not then provide the name, position and contact details of the appropriate person.

12. Is there a formal relationship between your agency and any others listed above? Provide copies of written agreements.

13. Does the State have the legislative power to ensure that all commercial shellfish growing areas have a marine biotoxin management plan and that ongoing biotoxin monitoring is routinely conducted? Provide a copy of title page of Act and other pages highlighting relevant sections.

14. Does the State have the legislative power to prohibit the harvesting of shellfish from aquaculture areas and wildstock resources, by both commercial and recreational fishers, due to unsafe environmental conditions caused by toxic algal blooms or due to an outbreak of shellfish poisoning? Provide a copy of the title page of Act and other pages highlighting relevant sections.

15. AQIS is responsible for ensuring that Australian food exports are safe and wholesome and in regards to shellfish they conduct regular audits of 'State Shellfish Quality Assurance Programs'. Provide copies of the biotoxin components of the two latest AQIS audits for your State and also the relevant biotoxin section of the latest USFDA audit (where appropriate). Are there certain types of shellfish or certain growing areas (from which no shellfish are to be exported) that AQIS has not investigated?

16. What agency in your State is responsible for ensuring that interstate shellfish currently sold on the domestic market in your State are free of biotoxins and are covered by a comprehensive biotoxin management plan incorporating a routine biotoxin monitoring program? Give name and contact details for relevant officer.

17. How many years has biotoxin monitoring been conducted in your State? Give a brief history (3-4 sentences) for each shellfish industry and for recreational harvesting by the general public.

18. Have there been any reports of illness caused by shellfish poisoning in your State? When, where, and what was the causative organism(s) and type of shellfish consumed?

19. List the number of growing area closures or harvesting restrictions that have been necessary due to toxic algal blooms and/or biotoxin contamination of shellfish. When (and for how long), where, what was the causative organism(s) and what shellfish were affected? Were public health alerts necessary on all occasions?

20. Provide a list of all toxic or potentially toxic algal species recorded to date in marine and estuarine waters in your State.

21. Apart from bivalve shellfish, are other types of seafood (e.g. fish, crabs, rock lobster, abalone) currently included in the State biotoxin program? What types of biotoxin analyses have been conducted in the past for such other seafood and what toxins (if any) have been detected in their tissues?

22. Do you receive all routine biotoxin monitoring data for the State in a timely manner, and is this data stored in a central file at your agency?

23. Who do you (the State shellfish QA program manager) consider should have overall responsibility and control for biotoxin management and biotoxin monitoring and assessment in your State?

24. Do you think that there is a need for a federal agency (existing or new) to implement the Australian biotoxin strategy, obtain necessary funding, produce national reports, disseminate information on toxic bloom events, latest monitoring and research results, etc. [A separate agency or personal submission on this aspect would be most appreciated.]

25. Provide a copy of *all* relevant biotoxin documents for the State that may be of value to the review team. These include biotoxin management plans (for State or individual shellfish growing areas), inter-agency agreements, AQIS and USFDA audit reports (biotoxin components), State task force reports or other biotoxin review documents, industry biotoxin reports/audits of individual programs, relevant State legislation, introduced pest/ballast water reports, etc.

Funding Issues

26. Provide an estimate of total funding for biotoxin management and biotoxin monitoring in 1998-99, 1999-2000 and current financial year. Give breakdown of salary (and full-time biotoxin staff equivalents) and operational costs excluding agency on-costs.

27. How is the biotoxin component of the State shellfish QA program funded? Provide a list of all funding sources (government and non-government agencies and shellfish industries) and an estimate of their percentage contribution for each shellfish industry in your State in the current financial year.

28. List all *potential* sources of funding and indicate the reason why you think each source should be included.

Biotoxin Management Plans

29. Do you have a comprehensive biotoxin management plan that sets out the management approach, relevant biotoxin and toxic algal standards, opening and closure criteria, etc. that applies to all State waters? Provide a copy of the latest plan.

30. What year was the latest plan produced? How often is this plan revised to incorporate new findings based on the latest local, Australian and overseas research and monitoring data?

31. Do you have specific biotoxin management plans developed for each shellfish growing area in the State based on a risk assessment conducted for each local environment? List and provide copies of all growing area plans. Who developed these plans (give required qualifications and experience of authors) and has any audit of the plans been conducted to validate the suitability and efficacy of the plan for the particular locality? In addition, have any audits been conducted to assess if the government or shellfish industry is conducting the biotoxin monitoring as documented? What qualifications are required for the two types of auditors?

32. Regarding the State biotoxin management plan(s):
Who collects the shellfish and algal samples?

    What toxin analyses are required? Specify methods for PSP, ASP, DSP, and NSP and provide example of result sheet from each laboratory.

What phytoplankton analyses are required? All species identified and recorded or only those named on list of potentially toxic species? Qualitative (perhaps relative abundance) or quantitative data? Describe methods and provide example of result sheet for each supplier.

What environmental data is collected at time of sampling?

[Note: if information is available in biotoxin plans then no need to answer here.]

33. Provide a list of all analytical and phytoplankton laboratories used to date and give contact details for each laboratory.

    Do these laboratories have NATA or any other accreditation for the specific analyses undertaken?

    Are the services provided satisfactory or is there a need for some improvement?

    Are there any required services that are not available in your State or within Australia?

34. Provide an additional list of all other possible service providers (with contact information) in your State.

A submission on any aspect of the Australian National Biotoxin Strategy from your own agency, any other relevant State agency, or any State shellfish industry would be most welcome. Could State program managers relay invitation and arrange mailing of returns. All submissions *must* be received by 20 October 2000.

Dr. Graeme Arnott (QualSafe Seafood Services) on behalf of Ms. Kirsten Todd (Cawthron Institute, Nelson, New Zealand), the Project Co-ordinator for the Australian National Biotoxin Strategy (11 September).

Appendix 2 Marine Biotoxin Laboratory Services Questionnaire

Marine Biotoxin Services

Questionnaire/ information request for providers of marine biotoxin services to assist in the development of an 'Australian National Biotoxin Strategy'.

Organisation/Staffing

1. Provide full name, address and contact details for both the parent organisation and biotoxin laboratory.

2. Describe the role and core skills of the biotoxin laboratory and the relationship between the laboratory and parent organisation where relevant.

3. Describe the facilities of the biotoxin laboratory and relevant facilities in other sections of organisation.

4. Does the biotoxin laboratory have NATA (or other) accreditation?

5. Who pays for the biotoxin analyses? Government agency and/or private clients?

6. Does your laboratory conduct marine biotoxin research in addition to routine commercial work?

7. List the key personnel (including 'manager') and their positions in the biotoxin laboratory. For each of the above staff provide a concise curriculum vitae (max. one page) including:

- Formal qualifications.
- Specific training in marine biotoxin analyses and toxic or harmful marine algae.
- Professional experience, especially with regard to marine biotoxin analyses and toxic or harmful marine algae.
- Attach relevant publications, particularly those related to the identification and analysis of marine biotoxins.

Methods/Services

8. What methods are in use in your laboratory? Provide references or a copy of the methods.

9. What compounds (the actual compounds not the class) are tested for routinely? [Refer to attached table/list of different compounds able to be tested in New Zealand.]

10. What compounds (the actual compounds not the class) are tested for by special request?

11. Are the tests validated? How?

12. Are the tests accredited/approved? Who by?

13. What standards are used? Are the standards obtained in house or externally (e.g. from NRCC)?

14. If conducting mouse bioassays are both the extraction procedure and mouse test performed in your laboratory?

15. What is the detection limit for each test? How are detection limits calculated?

16. Are positive results verified by repeating the test or conducting a different test? If the latter which test?

17. What sample size is used?

18. What is the turn-around time on samples for different tests?

19. How many marine biotoxin analyses would you conduct on average per week? What is the current weekly capacity of the laboratory?

20. How and in what form are results given to client? E-mail, phone or fax? Other?

21. Do you provide advice, e.g. a risk assessment, based on the results of routine analyses?

22. Do you provide any other biotoxin services? For example, do you prepare comprehensive marine biotoxin management plans or design testing programs for clients?

Please enclose any other relevant material about your organisation, or any comments regarding the biotoxin strategy, to the Project Team.

Send questionnaire responses and any other submissions to both Ms. Kirsten Todd and I at same time. E-mail replies are requested wherever possible. Contact details are as follows-

Dr. Graeme Arnott
QualSafe Seafood Services
11 Diggorra Court,
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Dr. Graeme Arnott (QualSafe Seafood Services, Victoria) on behalf of Ms. Kirsten Todd (Cawthron Institute, Nelson, New Zealand), the Project Co-ordinator for the development of the 'Australian National Biotoxin Strategy'.

Table of different compounds able to be tested in New Zealand:

Toxin	Test1	Test2	Test3	Test4
DSP				
OA	Mouse	PP2A	HPLC-FL	LCMS
DTX1-3	Mouse	PP2A	HPLC-FL	LCMS
PTX1-7	Mouse	HPLC-FL	LCMS	
YTX's	Mouse?	HPLC-FL	LCMS	
Gymnodimine	Mouse?	LCMS		
ASP				
Domoic acid	HPLC-UV	LCMS	ELISA	
PSP	Mouse	ELISA	MIST alert	HPLC-FL
NSP	Mouse	LCMS	ELISA	
Other				
AZP	Mouse?	LCMS		

? = may work on the test, unproven or poor response.

Table of all lipophilic compounds that may need to be tested:

DSP toxins	**NSP toxins**
Routine screen	**Routine screen**
OA	
DTX1	
DTX2	
DTX3 (7-O-acyl esters of OA and DTXs)	
Screened for if required:	**Screened for if required:**
DTX4	BTX-B1
DTX5	BTX-B2
OA diol esters	BTX B3
	BTX B4
	PbTx-3 (BTX-B skeleton)
Not tested	**Not tested**
	PbTx-1 (BTX-A skeleton)
	PbTx-2 (BTX-B skeleton)
	PbTx-10 (BTX-A skeleton)
	Hemi-BTXs A, B and C

Pectenotoxins	**Yessotoxins**
Routine screen	**Routine screen**
PTX2	YTX
PTX2sa	45-OH-YTX
Not tested	**Not tested**
PTX1	1-desulfoYTX
7 epi PTX2sa	45,46,47-trinor-YTX
PTX3	homo-YTX
PTX4	45-hydroxyhomo-YTX
PTX6	Adriatoxin
PTX7	

Miscellaneous toxins

Routine screen
Azaspiracids (AZ-1, 2 and 3)
Gymnodimine
Screened for selected species, locations, dates:
Ciguatoxin
Spirolides
"Wellington Hbr" toxin
3. Not tested
Coolia-toxin
Gymnodimine B
Goniodomin
Pinnatoxin
Prorocentrolides
Ostreocin (aqueous extraction, but a polyether)

Appendix 3 Marine Phytoplankton Laboratory Services Questionnaire

Marine Phytoplankton Services

Questionnaire/ information request for providers of marine phytoplankton services to assist in the development of an 'Australian National Biotoxin Strategy'.

Staffing

1. Who is in charge of the marine phytoplankton laboratory and what is their position?

    - Does this person supervise all marine phytoplankton services on a daily basis?
    - What percentage of their time is devoted to the provision of marine phytoplankton services?
    - What percentage of their time, if any, is devoted to the provision of freshwater algal services?
    - Is this person actively involved in conducting marine phytoplankton sample analyses?

2. How many staff are involved in the provision of marine phytoplankton services? List staff and their positions.

3. For all staff (including supervisor) provide a brief one page curriculum vitae including:
    - Formal education and qualifications.
    - Training in marine phytoplankton including any specific training in toxic and harmful marine algae. Include attendance at any of the international conferences on toxic phytoplankton.
    - Professional experience, especially with regard to the identification and enumeration of marine phytoplankton and toxic species in particular.
    - Key publications, particularly those related to marine phytoplankton.

Methods/Services

4. What methods and equipment do you use *routinely* to obtain quantitative estimates of species' cell concentrations? Provide references or copy of methods.

5. What other methods do you use, e.g. EM or genetic probes, and is the equipment available in your laboratory?

6. Do you conduct identifications on fresh or preserved samples (specify)?

7. On average, how many marine phytoplankton samples would you analyze per week? Give breakdown for qualitative samples (presence/relative abundance) and quantitative samples (species abundance in cells/L).

8. What is the turn-around time on samples? How long does it take to determine the cell concentration of a single species and all species?

9. What is the weekly capacity of the laboratory?

10. Do you have a list of toxic or harmful species (specify) which you use to determine if toxic species are present during routine sample analyses?

11. In what form is the data filed and what type of results do you issue routinely? Provide one-page examples of different result sheets.

12. How are the results given to client? E-mail, phone or fax? Other?

13. Do you provide other advice, e.g. a risk assessment, based on the results of routine analyses?

14. Who pays for the analyses? Government agency and/or private clients?

15. Does your laboratory have NATA or any other certification *specifically* for marine phytoplankton analyses? Does it have NATA certification for freshwater sample analyses?

16. Does your laboratory conduct marine phytoplankton research in addition to routine commercial work?

Please e-mail questionnaire responses and any other submissions to both Ms. Kirsten Todd and I at the same time. Contact details are as follows-

Dr. Graeme Arnott
QualSafe Seafood Services
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Dr. Graeme Arnott (QualSafe Seafood Services, Victoria) on behalf of Ms. Kirsten Todd (Cawthron Institute, Nelson, New Zealand), the Project Co-ordinator for the development of the 'Australian National Biotoxin Strategy'.

Appendix 4. Marine microalgae identification services available in Australia (n/s = not specified, n/a = not applicable)

Laboratory name		Micro-algal Services	Department of Primary Industry, Water and Environment	Water ECOscience	Dalcon Environmental Marine and Freshwater Consultants	Australian Government Analytical Laboratories	Sydney Water
State (from which response was received in the case of National organisations)		VIC	TAS	VIC	WA	VIC	NSW
NATA accreditation		x	x	✔	x	x	✔
Marine microalgae		✔	✔	✔	✔	✔	✔
Freshwater microalgae		x	✔	✔	n/s	n/s	✔
Water		✔	✔	✔	✔	n/s	✔
Sediment		✔	n/s	n/s	n/s	✔	n/s
Toxic species only		x	n/s	✔	x	✔	✔
All species		✔	n/s	✔	✔	n/s	✔
Qualitative analysis		✔	✔	✔	n/s	✔	✔
Quantitative analysis		✔	✔	✔	✔	n/s	✔
Cell Concentration		✔	n/s	✔	✔	✔	✔
Continuous –Flow Centrifugation		✔	x	x	x	x	x
Gravity Filtration		✔	x	x	✔	x	x
Sedimentation		✔	x	✔	x	x	✔
Sonicate and filter (for sediment)		✔	x	x	x	✔	x
Confirmatory method		✔	✔	✔	n/s	n/s	✔
Fluorescence microscopy		✔	x	x	x	x	x
Electron microscopy		✔	✔	✔	x	x	✔
Turn-around time		< 24 hours	<24 hours	<24 hours	n/s	5-10 days	<24 hours
Samples received	Net	✔	n/s		n/a		
	Van Dorn or hose	✔	n/s		n/a		
	Live	✔	n/s	✔	n/a	✔	
	Preserved	✔	n/s	✔	n/a	✔	
Number of samples		30	1/week	12-15	n/s	2-3/week	12-15
Sample capacity		60	20-40/week	12-15	n/s	n/s	12-15

Other Laboratory Services we are aware of offering this service in Australia:

South Australian Water Quality Centre
Hodgson Road
BOLIVAR SA 5110
Contact Mr W Emmett, Phone (08) 8259 0211; fax (08) 8259 0228
(listed on NATA website as being Accredited for 8.62 Aquatic Biology – marine systems. 01 Identification and enumeration of biota. Dinoflagellates.)

Vas Hosja, WA,
Aquatic Ecosystems Section
Water and Rivers Commission
phone 08 9278 0463
fax 08 9278 0586

Appendix 5 Marine biotoxin analytical services available in Australia

* Refers to whether biotoxin analytical services are offered commercially or as part of a research program. (n/s = not specified, n/a = not applicable)

Laboratory name	**Australian Government Analytical Laboratories (AGAL)**	**Water Toxin Analysis Services**	**Institute of Medical and Veterinary Science (IMVS)**	**Queensland Health Scientific Services**	**Australian Institute of Marine Science**	**State Chemistry Laboratory, Agriculture Victoria**	**Institute for Molecular Bioscience, University of Queensland**
State	VIC	NSW	SA	QLD	QLD	VIC	QLD
NATA accreditation	x	**x**	✔	**x**	**x**	✔	**x**
Dedicated Biotoxin lab	x	**x**	**x**	**x**	**x**	**x**	**x**
Commercial Laboratory*	✔	✔	✔	✔	**x**	✔	**x**
Research Laboratory*	**x**	✔	**x**	✔	✔	**x**	✔
TOXINS+METHOD							
PSP (STX, GTX, CTX etc)	x	**x**	✔	✔	✔	✔	**x**
mouse bioassay	x	x	✔	x	x	x	x
HPLC-FL	x	x	x	✔	✔	✔	✔
bioassay – (sodium channel and saxiphilin radio receptor assays)	x	x	x	x	✔	x	x
NSP/DSP screen (lipid soluble toxins)	**x**	**x**	✔	x	x	x	x
mouse bioassay	**x**	**x**	✔	x	x	x	x
DSP (OA, DTXs, PTXs, YTXs)	x	**x**	✔ (screen only)	✔	**x**	**x**	**x**
LC-MS	x	x	x	✔ (Gymnodimine method n/s)	x	x	x
ASP (domoic acids)	✔	**x**	**x**	**x**	**x**	✔	x
HPLC-UV	✔	x	x	x	x	✔	x
NSP (BTX'S)	**x**	**x**	✔ (screen only)	**x**	**x**	**x**	✔
LC-MS, LC-MS-MS, NMR, radioligand binding assay	x	x	x	x	x	x	✔
OTHERS	**x**	x	**x**	**x**	**x**	**x**	✔
AZP's	x	x	x	x	x	x	x
Spirolides	x	x	x	x	x	x	x
'Wellington Harbour' Toxin	x	x	x	x	x	x	x
Ciguatoxin	x	x	x	x	x	x	LC-MS, LC-MS-MS, NMR

Turn-Around Time	5-10 days	3 days	2-3 days (PSP), NSP/DSP screen >1 week)	2-3 days	1-2 days (HPLC), 1 day (radio-receptor assays)	1-5 days	3-12 months
Advice	**x**	Limited	✔	**x**	**x**	**x**	✔
Standards from:	DACS-1B, NRCC	Commercially available microcystin	USFDA	n/s	STX – Tohoku uni.	DA – NRCC PSP – NRCC, Oshima	x
Number of samples	1-2/week	50/year	8-10/week	1-2	As per research program	2/week	All research
Capacity of lab	n/s	Dependent on demand	Close to limit	10-20	HPLC – 1-2/day. Radio receptor assay – 100/day	100/week	n/a

PART B

Australian Marine Biotoxin Management Plan for Shellfish Farming

A model Australian marine biotoxin management plan

PART B – A MODEL AUSTRALIAN MARINE BIOTOXIN MANAGEMENT PLAN

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1 AMENDMENTS

Authors note: Details in this plan in *italics* are to be filled in by the Program Manager for each State and Territory. This is a model plan for Australia and is applicable to both cultured and wild harvested shellfish.

Amendments can be made to this plan by contacting the coordinator with the suggested changes and reasons for the change. To become part of this plan, amendments need to be issued with an amendment form. Amendments are numbered in sequence.

Amendments are identified by the issue number in the page header, on the contents page and by the symbol * in the left margin adjacent to the line which has been changed.

The coordinator of this plan is:

(Enter name of coordinator, maybe chairperson of ASQAAC, or a state program manager willing to take responsibility for it.)

1.1 Amendments Record

It is important this plan is kept up to date by the prompt incorporation of amendments.

To update the plan, remove the appropriate pages, destroy them and replace with the newly issued pages. Instructions will be included in the covering letter when amendments are issued and sent. File the covering letter at the back of the plan and sign off and date this page.

Issue No.	Date	Initials

2 INTRODUCTION

2.1 Background

Some species of marine microalgae (phytoplankton) produce natural toxins which when filtered by shellfish (e.g. oysters, mussels, scallops and clams) can be concentrated to levels which are harmful to humans consuming the shellfish causing toxic shellfish poisoning (TSP). There are four shellfish poisoning syndromes: Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP) and Amnesic Shellfish Poisoning (ASP). See Appendix 10 for a list of the causative organisms of these poisoning syndromes.

TSPs pose a risk to consumers of shellfish both from commercially and recreationally obtained shellfish. The risks are not only health risks to consumers, but there is also the potential damage to consumer confidence and export trade. These risks can be managed by marine biotoxin management plans.

2.2 Aims and Objectives

The principle aim of this marine biotoxin management plan is to ensure the protection of shellfish consumers from the hazards of marine biotoxin poisoning. This plan is equally relevant to cultured and wild harvest shellfish (both commercial and recreational).
In order to meet this aim, the following objectives have been established:

- The maintenance of a monitoring program using both phytoplankton monitoring and shellfish toxin testing in conjunction with one another. Phytoplankton monitoring is used to provide an early warning of the potential for contamination of shellfish with marine biotoxins, however shellfish testing is used to make harvesting and regulatory decisions;
- The harvest of shellfish which are free from marine biotoxins;
- An effective and co-ordinated response to marine biotoxin events to minimise the risk of human illness;
- The management of information to the media to ensure public awareness and to minimise potential adverse publicity to the shellfish industry; and
- The maintenance of contingency plans to allow fast response in an event.

2.3 Scope

This marine biotoxin management plan is designed primarily for, and is equally applicable to, both aquaculture and commercial wild shellfish harvesting, but could also be implemented by responsible agencies for the protection of recreational gatherers.
This plan covers molluscan bivalve shellfish.

2.4 Review

This plan will be reviewed as appropriate and at least on an annual basis. This review shall be undertaken by an agency with good oversight of the marine biotoxin management program, and with knowledge of each state.

2.5 Abbreviations and Acronyms

TSP	Toxic shellfish poisoning
ASP	Amnesic shellfish poisoning
DSP	Diarrhetic shellfish poisoning
PSP	Paralytic shellfish poisoning
NSP	Neurotoxic shellfish poisoning
AQIS	Australian Quarantine and Inspection Service
(*STATE)*SQAP	*(STATE)* Shellfish Quality Assurance Program
ASQAP	Australian Shellfish Quality Assurance Program
ASQAAC	Australian Shellfish Quality Assurance Advisory Committee
NATA	National Association of Testing Authorities, Australia
ELISA	Enzyme Linked Immuno-Sorbent Assay
HPLC	High Performance Liquid Chromatography
Health	*Insert name of State Department responsible for public health here*
Food Safety	*Insert name of State Department responsible for food safety here*
Fisheries	*Insert name of State Department responsible for fisheries here*
Environmental Health	*Insert name of State Department responsible for environmental health here*
Aquaculture	*Insert name of State Department responsible for aquaculture here*
Industry	*Insert Industry name(s) here*
MU	Mouse Units

2.6 Definitions

Authorised Officer *(or other appropriate title)* – an officer employed to perform specified duties to ensure the requirements of the *(State)*SQAP are complied with.

3 ADMINISTRATION

3.1 Legislation

3.1.1 National

- Australia New Zealand Food Standards Code 1992
- Export Control Act 1982
- Export Control (Processed Food) Orders 1992
- Australian Shellfish Sanitation Control Program Operations Manual
- *Insert any other appropriate acts*

3.1.2 State

- Health Act
- Fisheries Act
- Food Act
- *Insert any other appropriate acts*

3.2 Roles and Responsibilities

3.2.1 Fisheries

State the role and responsibility of this state department here. Does this department have the authority to:

- *issue aquaculture licences and leases;*
- *control the harvesting of shellfish based on sanitary conditions;*
- *have the oversight of the sampling program;*
- *perform and/or supervise sampling;*
- *ensure no illegal harvesting takes place when a closure is in place;*
- *retain records of aquaculture licences and conditions, closure and re-opening notices of lease sites;*
- *perform survey and classification of shellfish growing areas;*
- *control post harvesting and transport of shellstock;*
- *detain and recall product considered unfit for human consumption;*
- *enforce necessary sanitary controls for processing plants and vehicles handling shellstock; and*
- *maintain epidemiological data for notifiable diseases (including TSP cases).*

3.2.2 Health

State the role and responsibility of this state department here. Does this department have *the authority to:*

- *issue aquaculture licences and leases;*
- *control the harvesting of shellfish based on sanitary conditions;*
- *have the oversight of the sampling program;*
- *perform and/or supervise sampling;*
- *ensure no illegal harvesting takes place when a closure is in place;*
- *retain records of aquaculture licences and conditions, closure and re-opening notices of lease sites;*
- *perform survey and classification of shellfish growing areas;*
- *control post harvesting and transport of shellstock;*
- *detain and recall product considered unfit for human consumption;*

- *enforce necessary sanitary controls for processing plants and vehicles handling shellstock; and*
- *maintain epidemiological data for notifiable diseases (including TSP cases).*

3.2.3 Food Safety

State the role and responsibility of this state department here. Does this department have *the authority to:*

- *issue aquaculture licences and leases;*
- *control the harvesting of shellfish based on sanitary conditions;*
- *have the oversight of the sampling program;*
- *perform and/or supervise sampling;*
- *ensure no illegal harvesting takes place when a closure is in place;*
- *retain records of aquaculture licences and conditions, closure and re-opening notices of lease sites;*
- *perform survey and classification of shellfish growing areas;*
- *control post harvesting and transport of shellstock;*
- *detain and recall product considered unfit for human consumption;*
- *enforce necessary sanitary controls for processing plants and vehicles handling shellstock; and*
- *maintain epidemiological data for notifiable diseases (including TSP cases).*

3.2.4 Australian Quarantine and Inspection Services

AQIS is the national government agency responsible for the administration of the export controls for seafood. The agency administers the export inspection system and provides certification for shellfish exports.

AQIS administers the export inspection program, which includes provision for:

(a) the registration of premises, including vehicles, which prepare shellfish intended for export;

(b) the inspection of registered establishments for implementation of good food processing practises;

(c) conducting Hazard Analysis Critical Control Point (HACCP) based food processing controls.

AQIS staff conduct compliance inspections and audits of land based shellfish processing establishment in accordance with the compliance history of the establishment and food safety risk associated with the food being prepared for export. The *Export Control (Processed Food) Orders 1992* also regulate the application controls for shellfish handling, processing, purification, packing, storage, shipping, the labelling of shellstock to enable source identification and the recall, detention, seizure or destruction of shellfish unfit for human consumption.

AQIS also provides an audit role for the *(STATE)*SQAP (including the marine biotoxin management plan).

3.2.5 Industry

State the role and responsibility of the associated industry here. Does the industry:

- *have the oversight of the sampling program;*
- *perform and/or supervise sampling;*
- *ensure no illegal harvesting takes place when a closure is in place;*
- *any other roles the industry takes*

3.2.6 Australian Shellfish Quality Assurance Advisory Committee

The role of the Australian Shellfish Quality Assurance Advisory Committee (ASQAAC) is to provide a national overview of shellfish safety and quality, and to provide a set of minimum requirements agreed to by all states and territories (refer to the ASQAAC terms of Reference).

3.2.7 Other relevant agencies

Add other relevant agencies here e.g.

- *Seafood Services Australia*

3.3 Local Marine Biotoxin Management

3.3.1 Each shellfish growing / harvesting area shall have a marine biotoxin management plan, which contains:

- agency and personnel contact details at local and state levels;
- the marine biotoxin sampling sites for each commercial shellfish growing area (including maps showing the shellfish growing /harvesting areas and sampling sites within these);
- the frequency of shellfish and phytoplankton monitoring for each growing area;
- procedures for phytoplankton and shellfish sample collection and dispatch;
- early warning indicators;
- contingency plans;
- procedures for notification of results to industry and others;
- procedures for harvesting area closure and re-opening;
- procedures for detention and recall of harvested product;
- draft media statements;
- surveillance procedures for closed areas;
- the laboratories used, which should have ISO17025 accreditation.

4 SAMPLING

4.1 Sampling Site Selection

4.1.1 When establishing sampling sites for toxic phytoplankton and shellfish there are general factors that need to be considered:

- The history of phytoplankton and marine biotoxin activity in the area.
- The need to cover all major commercial and recreational shellfish harvesting areas.
- The need to sample seasonal fisheries immediately prior to and during their open season.
- The accessibility of sample sites in all weather conditions
- Environmental factors likely to influence sampling, such as:
    - major currents,
    - retention zones and circular patterns;
    - areas where algal blooms and fish kills are regularly observed, or have been regularly observed in the past;
    - areas where rivers have a major impact;
    - impact of drains and concentrated point sources of ground water seepage;
    - any other factors that may have influence sampling.
- The routine sampling sites will be able to be changed if the needs of the monitoring program change.
- Sampling sites should be located where past experience has shown marine biotoxins are most likely to appear first.

4.1.2 Specific criteria for the selection of phytoplankton sampling sites:

- Sites are chosen so that the water being sampled is representative of the water being filtered by the shellfish being monitored.
- Consideration should be given to the tidal stage to ensure that samples collected represent the water the shellfish are about to filter rather than the water they have already filtered.
- For shellfish on line culture, the water samples should be collected so the entire depth of lines are sampled.
- For intertidal culture, sampling may be around the lease area at high tide or at the change of tide.
- For wild harvest, sampling should be such that the water consumed by shellfish is sampled. Phytoplankton monitoring may be ideal for use for wild harvest dredging of species (e.g. scallops)
- Samples should not be collected from the surf for two reasons:
    (i) fragile cells (such as *Karenia brevis* (=*Gymnodinium breve*)) can be damaged making identification difficult or impossible;
    (ii) samples contain a lot of silt which makes accurate identification difficult or impossible.
- Sample collection using bottles and tubes/hoses should be used in preference to nets for 2 reasons:
    (i) net sampling breaks up fragile cells such as *Karenia/Gymnodinium* species, giving a false picture of the true structure of the phytoplankton community;
    (ii) net sampling gives only qualitative results, and management decisions need to be based on quantitative results which can be achieved by using sampling methods such as bottle and tube/hose sampling.

- For intertidal culture, sites may need to be in deeper channels, but should still be collected so they are representative of the water the shellfish are filtering.

4.2 Long-term Goals

4.2.1 Sampling for the monitoring program needs to be designed with the following long-term goals in mind:

- Establishment of a long-time data set of routine results, marine biotoxin events and associated ecological factors (see 4.7); this will allow better risk assessment, analysis of trends and prediction of marine biotoxin events.
- Early warning of potential marine biotoxin contamination by highlighting changes in abundance of potentially toxic phytoplankton species.
- Determination of population dynamics of toxin producing phytoplankton species during marine biotoxin contamination events; including the variation in numbers through a growing area and the influence of tidal and diurnal cycles on abundance.
- Increased knowledge and a wider understanding of those species that pose a potential marine biotoxin threat to commercial and recreational harvesters of shellfish; this will allow a better understanding and application of risk assessments.

4.3 Sampling Frequency

4.3.1 Phytoplankton sampling should be undertaken frequently and regularly, with the frequency remaining constant throughout the year, as potentially harmful species can occur at any time of the year. Internationally, weekly phytoplankton sampling is the norm.

If less frequent monitoring is implemented, it must be accepted that this may mean some bloom events are missed and therefore the use of phytoplankton monitoring as an early warning is compromised.

4.3.2 Shellfish sampling should also occur regularly in conjunction with the phytoplankton sampling, and harvesting and regulatory decisions made based on these results. Depending on the history of the area, sampling may take place less frequently, maybe fortnightly or monthly.

4.3.3 In some cases sampling frequency may be less frequent (e.g. in seasonal fisheries), and may be increased in response to results of the regular monitoring program.

4.4 Shellfish Sample Species

4.4.1 Shellfish samples should be of those species that are most likely to reveal the early presence of marine biotoxins and which are most likely to show the highest toxin levels. The following criteria may be helpful in selecting sampling species:

- Some shellfish species are better indicators of marine biotoxins than others. Internationally mussels are used as the sentinel species of choice because they generally indicate marine biotoxins before other species.
- Scallops appear to be more sensitive to domoic acid then some other species of shellfish, this should be taken into account when including these species in a Marine Biotoxin Management Plan.
- Closures may be made on a species by species basis (see 6.1.3 for further information).

4.5 Phytoplankton Species to Monitor For

4.5.1 Appendix 10 contains a list of phytoplankton species present or likely to be present in Australian waters sorted into the following categories:

- Category A - Species known to be present in Australian waters and proven to produce toxins either in Australia or internationally.
- Category B - Potential toxin producing species (i.e. toxicity untested/unclear) known to be present in Australian coastal waters.
- Category C - Other potential toxin producing species world-wide that may be present in Australian waters.

4.5.2 Appendix 12 lists the trigger levels for phytoplankton species. These relate to an integrated sample collected either with a tube/hose sampler, or by a series of discrete depth samples. If these levels are exceeded in any one depth, further testing should also be undertaken.

4.6 Sample Size

4.6.1 Phytoplankton - Water samples should be of sufficient volume to allow the testing to be carried out with sample to spare. In general, 500 ml to 1 litre is collected. This may be by discreet depth samples (e.g. with a van Dorn bottle); as a column sample (e.g. with a tube/hose sampler); or as a grab sample. The volume of sample collected will depend on the site and past and current practices.

4.6.2 Shellfish - samples should be of sufficient size to allow testing for all marine biotoxin groups if necessary and to give a good random sample of shellfish from the site. The minimum number of shellfish to be collected is 12, and should allow approximately 400 g of shellfish flesh removed from the shell.

4.7 Environmental Information

4.7.1 It is generally recommended to obtain some environmental parameters at the same time. Some types of data that may be collected are:

- Physical data: salinity, water temperature, visibility (by secchi disc or sampling bottle is a good alternative).
- Meteorological: river runoff, rainfall, wind speed and direction, irradiance.
- Nutrient: inorganic nitrogen, phosphorus.

4.8 Sampling Safety

Sampling officers should carry out sampling in a safe manner. Safety issues shall be addressed in individual growing area management plans.

4.9 Sampling Officers

The Authorised Officer is responsible for training of sampling officers, to ensure accuracy and consistency of sample collection across the program. The Authorised Officer shall have a good understanding of marine biotoxins and related issues.

Sampling officers shall be trained in sampling for shellfish and phytoplankton, by the Authorised officer, and audited regularly.

4.10 Sample Handling/Care

4.10.1 Phytoplankton: Water samples should be transported in a manner which reduces major temperature changes. They should not have ice added during transport, they should not be refrigerated and they should not be allowed to heat up (e.g. by leaving them in the sun).

Samples should be collected so as to leave a small air space in the sample bottle.

4.10.2 Shellfish: Shellfish samples should be transported so they arrive at the testing laboratory within 24 hours of collection, alive and in good condition.

They shall be kept cool, packed with ice to maintain a temperature of 10°C or less.

Samples should not be frozen.

For some shellfish species (e.g. Sydney Rock Oysters) this temperature may be inappropriate. In instances like these, temperature limits should be set such that the shellfish arrive in the laboratory live and in good condition.

5 MONITORING

5.1 Routine Marine Biotoxin Monitoring

The routine frequency of marine biotoxin monitoring shall be stated in the individual growing areas marine biotoxin management plan.
In general it should have frequent and regular (i.e. weekly) phytoplankton monitoring, complemented by shellfish flesh testing also on a regular basis, and also when indicated by routine phytoplankton monitoring results i.e. when pre-set action levels (Appendix 12) are exceeded. Phytoplankton sampling should be performed with the aim of producing quantitative results. Therefore samples should be collected using either bottle samplers or tube/hose samplers. Net hauls may also be used to collect qualitative information, however quantitative information should be used to make management decisions.

5.2 Contingency Plan for Marine Biotoxin Events

5.2.1 This contingency plan will be instigated in any of the following scenarios:

- Presence of phytoplankton species above the levels stated in Appendix 12.
- Presence of any phytoplankton species known to be a toxin producer internationally, but not previously observed or tested in Australian waters.
- Investigation of areas where marine biotoxin levels are increasing but may not have exceeded a regulatory limit (Appendix 13).
- To monitor the movement of toxic phytoplankton species between growing areas.

5.2.2 During a closure event, the closed area should be thoroughly investigated to establish the species affected and the spatial extent of toxicity. Therefore potentially allowing re-opening for specific shellfish species and re-defining the extent of the closure.

5.2.3 Laboratory services

Appendices 3 and 4 list the organisations that can provide analytical services for phytoplankton analysis of water samples and biotoxin analysis of shellfish flesh.

Results need to be communicated to the relevant parties in a timely manner. For phytoplankton results, this should be within 24 hours of receipt of sample by the laboratory. For shellfish samples this should be within 2-3 days of receipt of sample, but is dependent on the test being performed, and whether the test is routine or urgent.

5.2.4 Result reporting and notification

A communication network needs to be established to ensure that there is clear flow of communication between all parties i.e. farmers, processors, *(STATE)*SQAP managers, laboratories, health officials, fisheries officials, regulators, adjacent states and other responsible parties.

For results that exceed regulatory flesh limits (Appendix 13), the laboratory shall notify the Authorised Officer immediately by telephone, and follow this with a confirmatory facsimile or e-mail.

For phytoplankton results exceeding levels above the action levels in Appendix 12, or which the laboratory considers of note, the laboratory shall notify the Authorised Officer immediately by telephone, and follow this with a confirmatory facsimile within 1 hour.

6 AREA CLOSURE AND RE-OPENING

6.1 Mechanism for Closure and Re-opening

6.1.1 The Authorised Officer will close a shellfish growing area to harvesting and the movement of all shellfish immediately that any criteria in section 6.2 are met.

6.1.2 The closure area will extend to the nearest sample site below regulatory closure level or at the discretion of the SQAP manager.

6.1.3 Closures may be made on a species-specific basis due to differences in shellfish accumulating toxins. Each species should be tested to determine the toxin levels.

6.1.4 Where a commercial area is included in a closed area, the closure notice will be faxed, posted, e-mailed or contact made by phone to all growers, industry representatives; AQIS; *enter name of State Department responsible for public health here; enter name of State Department responsible for food safety here; enter name of State Department responsible for fisheries here; enter name of State Department responsible for environmental health here; enter name of State Department responsible for aquaculture here.*

6.1.5 A backdated recall of commercial product should be made (refer to section 8.1).

6.2 Closure Criteria

The following criteria determine whether a closure needs to be put in place:

6.2.1 Marine biotoxins are present in shellfish in levels over the regulatory levels in Appendix 13;

6.2.2 Cases of human illness consistent with the case definitions for PSP, NSP, DSP and ASP (Appendix 11) have resulted from the consumption of shellfish from a particular area;

6.2.3 The Authorised Officer determines a closure is necessary for any other reasons (e.g. toxins present in neighbouring areas, potential toxin producing phytoplankton species which have not previously been recorded are present in the area).

6.3 Industry Instigated Closure

Industry may choose to instigate a voluntary closure based on criteria such as toxins in neighbouring areas, rising levels of toxin in shellfish, rising levels of toxic phytoplankton, or any other criteria deemed important enough to necessitate a closure.

6.4 Re-opening Criteria

A shellfish growing area closed due to marine biotoxins shall not be reopened until the Authorised Officer has determined that each of the following requirements for reopening have been adequately addressed.

6.4.1 Results from the edible portion of shellfish flesh from representative sites in the closed area shall meet the following criteria:

(i) Paralytic shellfish poisoning (PSP), less than 80 µg of saxitoxin equivalent /100 g of edible portion of shellfish flesh, by mouse bioassay with a maximum observation time of 1 hour, in three consecutive samples of the same species from initially positive sample site, taken over a minimum period of 14 days,

i.e. The first sample on day 1, the second after 1 week and the third no earlier than day 14;

(ii) Neurotoxic shellfish poisoning (NSP), less than 20 mouse units/100 g of edible shellfish flesh, by ether extraction and mouse bioassay with a maximum observation time of 6 hours, in two consecutive samples of the same species from the initially positive sample site, the second of which must be taken no earlier than 2 days after the taking of the initial clear sample;

(iii) Amnesic shellfish poisoning (ASP), less than, 20 ppm (µg/g) of domoic acid in the edible shellfish flesh, by high performance liquid chromatography (HPLC), in three consecutive samples of the same species from initially positive sample site, taken over a minimum period of 14 days, i.e. The first sample on day 1, the second after 1 week and the third no earlier than day 14;

(iv) Diarrhetic shellfish poisoning (DSP), less than 20 µg/100 g of edible shellfish flesh (approx. 5 mouse units) by 24 hour mouse bioassay, in two consecutive samples of the same species from the initially positive sample site, taken not less than 7 days apart.

6.4.2 The level of toxic phytoplankton relating to the toxin has shown a clear downward trend and the cell counts are below the limit in Appendix 12 to initiate closure. The Authorised Officer should consider and judge if the level of other potentially toxic phytoplankton species are increasing and therefore will not necessitate another closure within a short timeframe.

6.4.3 Once below the regulatory limit, toxin levels shall be decreasing or static in consecutive samples in order for the area to be re-opened.

(i) No cases of human illness, notified to the Health Authorities and consistent with accepted case definitions (Appendix 11) for PSP, NSP, ASP, or DSP, shall have resulted from the consumption of shellfish harvested since the date of collection of the first clearance sample from within or adjacent to the closed area.

(ii) Shellfish from adjacent areas shall be sampled and the results shall have been evaluated for their relationship to the area to be opened. Toxin levels shall be decreasing or static in adjacent areas. This may involve contacting adjacent Authorities to assess the impact of the marine biotoxin contamination in or on coastal areas under their jurisdiction.

(iii) The hydrography of the area and the pattern of toxicity at sample sites shall have been considered in assessing the potential of a re-occurrence of the toxicity.

(iv) All major shellfish harvesting areas in the area to be open shall have been represented by the spread of sampling sites.

(v) The types of shellfish sampled from the area shall be representative of those species normally harvested from the area.

(vi) The density of potentially toxic phytoplankton species shall be proportionally related to the overall phytoplankton community and the plankton transport and retention currents, where this information is available.

(vii) Other conditions or limitations may be imposed if considered necessary by the Authorised Officer.

(viii) The Authorised Officer shall, on each reopening event, prepare documents including the data, environmental conditions and factors leading to the decisions.

6.4.4 Resumption of harvest shall be accompanied by increased monitoring for at least 4 weeks.

7 INVESTIGATION OF ILLNESS DUE TO TOXIC SHELLFISH POISONING

7.1 Notification

All suspected cases of toxic shellfish poisoning are notifiable as cases of suspected food borne illness to *(enter name of responsible agency here).* It is the responsibility of the *(enter name of responsible agency here)* to ensure that general practitioners are aware of the need to notify suspected cases so that these can be followed up.

7.2 Investigation

Where toxic shellfish are suspected of being the cause of an illness, it is the responsibility of the *(enter name of responsible agency here)* to determine the source of the contamination and the method of handling the shellfish.

TSP investigations should be undertaken in a timely manner and using sound epidemiological principles. This will ensure that valuable information is gained so that TSP events in Australia are better understood. As with the aim of any epidemiological investigation the aim is control and prevention of further TSP episodes.

All suspected cases of TSP should be investigated. The investigation should include the following foundation steps:

(i) Verifying the diagnosis of report cases, and identify the specific etiologic agent responsible.
(ii) Confirm that an outbreak exists. Check for other cases at appropriate points e.g. boating clubs, medical practices in area.
(iii) Describe the cases in the epidemic or outbreak according to the variables of time, place and person.
(iv) Identify the source of the agent and its mode of transmission, including the specific vehicles, vectors and routes that may have been involved.
(v) Identify the populations that are at an increased risk of exposure to the agent.
(vi) Plan and implement control measures – close harvest areas, issue warnings, undertake recalls, etc.
(vii) Evaluate the control measures.

The sequence of these objectives indicates the sequence in which the logic proceeds in an epidemiologic investigation, but it is not necessarily the sequence in which the investigation itself is conducted. In practice, several steps of the investigation may be in progress simultaneously e.g. shellfish samples should be taken as soon as there is evidence of a problem.

Of importance in the investigation is the establishment of a "case definition" – a broad definition will ensure high sensitivity in finding potential cases, but is very likely to collect false positive cases. A case definition that is too narrow is likely to miss positive TSP cases.

Interview techniques are important to ensure that the information is factual, consistently collected and not affected by biases.

A copy of a model case investigation form is to be included in Appendix 14.

7.3 Immediate Action to be Taken in Suspected Toxic Shellfish Poisoning Cases

7.3.1 Restrictions – where investigation suggests that toxic shellfish may be the cause of illness, an immediate closure should be placed on harvesting by commercial and recreational harvesters pending the results of more detailed investigations.

7.3.2 Closures of harvesting areas – should be accompanied by immediate additional sampling of both shellfish and water in the affected area to determine the levels and size of the area affected. The level of toxins in the shellfish must be determined in order to define the closure area. Harvesting must cease until regular monitoring demonstrates that the re-opening criteria has been met (Section 6.4).

7.3.3 Control of movement of harvested shellfish – It is the responsibility of (*enter name of responsible agency*) to undertake a product recall/detention as in Section 7 with the co-operation of the appropriate responsible agencies (*enter name of State Department responsible for public health here, enter name of State Department responsible for food safety here, enter name of State Department responsible for fisheries here, enter name of State Department responsible for environmental health here, enter name of State Department responsible for aquaculture here*) and industry.

7.3.4 Notification – Notices shall be placed in prominent places near harvesting areas advising the public of the closure and to advise against consuming shellfish from within the closed area. This notification is the responsibility of (*enter name of responsible agency here*).

7.3.5 Communication – liaison between all appropriate organisations and individuals will be established to ensure that investigations are well co-ordinated. The organisations and individuals may include:

- *enter name of State Department responsible for public health here,*
- *enter name of State Department responsible for food safety here,*
- *enter name of State Department responsible for fisheries here,*
- *enter name of State Department responsible for environmental health here,*
- *enter name of State Department responsible for aquaculture here*
- industry
- AQIS
- Phytoplankton laboratory representative
- Biotoxin laboratory representative

7.3.6 Sampling - Samples should be taken where available and may include remains of meals, samples of commercial product from the same batches of product as consumed and samples taken from the suspected harvesting areas.

Samples need to be of sufficient size to allow analysis for non-marine biotoxin sources of illness (such as bacterial, viral or chemical contamination) to be eliminated.
If microbiological testing is required, the sample shall be transported in such a way as to prevent contamination, and identified appropriately.
For cases showing gastro-intestinal symptoms, faecal samples should be requested to eliminate bacterial/viral causes of illness.

7.3.7 Funding - Investigation of toxic shellfish poisoning incidents and the associated sampling is funded by (enter name of responsible agency here).

8 PRODUCT CONTROL

8.1 Product Recall

When harvesting or growing areas are closed due to the presence of marine biotoxins, product may need to be recalled or detained. This recall or detention will be backdated to, and including, the day following the last sample date with marine biotoxin results below the regulatory limit (Appendix 13).

Product recall is the responsibility of the growers, manufacturers, processors, distributors and retailers of affected product, in conjunction with regulators.

The recall shall be instigated within 24 hours of the harvest area closure.

8.1.1 Domestic recall

For a recall of domestic product the following procedure is followed:

(i) Industry are advised to immediately cease harvesting, processing, distribution and sales by the *(STATE)*SQAP Manager or Authorised Officer.

(ii) *(STATE)*SQAP Manager or Authorised Officer advises ANZFA, *Fisheries, Health, any other state organisations required to be informed* of the full particulars of the shipment.

(iii) If considered appropriate, a media statement will be made by *Health, Fisheries* advising the public. If not considered appropriate, Industry will place a Recall Notice in the relevant newspapers.

(iv) The grower(s), manufacturer(s), processor(s), distributor(s) and retailer(s) are advised of all details necessary for them to identify and withdraw product from sale.

(v) The return or disposal of the contaminated product is to be arranged by a competent independent authority in an approved sanitary site.

(vi) A monitoring program or sampling program may be undertaken to determine the extent of the problem and test if the product is acceptable for release.

(vii) A detailed summary recall report outlining the full scope of the recall, and the eventual outcomes is signed off by the *(STATE)*SQAP Manager or Authorised Officer, and is provided to ANZFA, *Fisheries, Health, any other state organisations required to be reported to.*

8.1.2 Export recall

For a recall of export product the following procedure is followed:

(i) Industry are advised to immediately cease harvesting, processing, distribution and sales by the *(STATE)*SQAP Manager or Authorised Officer.

(ii) *(STATE)*SQAP Manager or Authorised Officer advises ANZFA, AQIS, *Fisheries, Health, any other state organisations required to be informed* of the full particulars of the shipment.

(iii) The grower(s), manufacturer(s), processor(s), distributor(s) and retailer(s) are advised of all details necessary for them to identify and withdraw product from sale.

(iv) The return or disposal of the contaminated product by the Health Authority in the country of destination is co-ordinated through AQIS by the Industry Representative.

(v) A monitoring program or sampling program may be undertaken to determine the extent of the problem and test if the product is acceptable for release.

(vi) A detailed summary recall report outlining the full scope of the recall, and the eventual outcomes is signed off by the *(STATE)*SQAP Manager or Authorised Officer, and is provided to ANZFA, AQIS, *Fisheries, Health, any other state organisations required to be reported to.*

8.1.3 Notification to consumers

Where product has gone beyond the distribution chain to consumers, the consumers may need to be warned. This should be considered part of the recall process. For an effective recall, advertising should occur in all areas where the product is distributed. This may require media releases or paid advertising in newspapers, on radio or on television.

This shall occur within 24 hours of an area closure.

8.1.4 Detained product

Shellfish and shellfish products should be held by the processor until biotoxin sample results from the area show that levels are below regulatory limits or negative.

8.2 Product Traceability

8.2.1 All sales to restaurants and retail outlets (domestic and export) must be traceable to the farm. All packaging carries an identification label or tag in accordance with the following procedures:

(i) A durable, waterproof tag is affixed to each container of shellfish by the harvester, showing the following information:
- the name of the licence holder;
- the unique number of the licensed site;
- the name of the harvesting area;
- the date of harvest;
- the type and quantity of shellfish (eg mussels (10kg)).

(ii) The tag is applied to a container of shellfish at the time of harvest once the shellstock are cleaned.

(iii) These details are inscribed on all documentation and packaging to the final point of consumption and accompany individual consignments.

8.2.2 All industry members maintain effective record keeping, showing information on date of sale, quantity and distribution. This information can be made available on request to the (STATE)SQAP Manager or Authorised Officer or Fisheries. Records of all customer complaints are also maintained.

9 REFERENCES

Andersen, P. 1996. Design and Implementation of Some Harmful Algal Monitoring Systems IOC Technical Series No. 44, UNESCO

Biotoxin Management Plan. 2000. Tasmanian Shellfish Quality Assurance Program.

Marine Biotoxin Management Plan. 2000. South Australian Shellfish Quality Assurance Program

National Marine Biotoxin Management Plan. 1996. New Zealand Marine Biotoxin Management Board, New Zealand

Phytoplankton Manual. 1978. UNESCO.

Western Australian Shellfish Quality Assurance Program - Operations Manual. 1999.

Appendix 1 Contacts

List relevant contacts here:

State Program Manager
Fisheries Representatives
Health Representatives
Food Safety Representatives
Environmental Health Representatives
AQIS Representatives
Laboratory Contacts
Any other people.

Appendix 2 Communication Network Diagram and Responsibilities

Insert schematic diagram of information flow, list of responsibilities of government departments, and others

Appendix 3 Approved Laboratories for Phytoplankton Enumeration and Identification

Insert names, addresses and contacts for laboratories approved for marine phytoplankton enumeration and identification. At a minimum these labs should have NATA accreditation specifically for marine phytoplankton, and should have staff who have attended international training courses such as those run by the IOC.

Appendix 4 Approved Laboratories for Marine Biotoxin Analysis of Shellfish Flesh

Insert names, addresses and contacts for laboratories approved for marine biotoxin analysis. At a minimum, these labs should have NATA accreditation.

Appendix 5 Sampling Sites

Insert table of sampling sites including grid references

Appendix 6 Sampling Officers

Insert names, addresses and contact details of approved sampling officers

Appendix 7 Marine Biotoxin Analytical Methods

Paralytic Shellfish Poison (PSP)

Reference:

Enter reference details here

Amnesic Shellfish Poison (ASP)

Reference:

Enter reference details here

Neurotoxic Shellfish Poison (NSP)

Reference:

Enter reference details here

Diarrhetic Shellfish Poison (DSP)

Reference:

Enter reference details here

Appendix 8 Marine Biotoxin Monitoring Program Sample Collection Form

Insert a copy of the form to filled out and accompany samples to the laboratories.

Appendix 9 Phytoplankton Sampling Procedures

Collecting Phytoplankton samples using the tube/hose sampler

Equipment: **Tube/hose sampler**

Clean bucket - rinse with sea water
Sample bottles - 2 for each sample taken
Lugols Iodine for preserving one of the samples
Polystyrene bins for transporting samples

Method:

Prepare tube/hose	Remove bung from end
Collect sample	Lower weighted end first Hold top end securely Lower very slowly to maximum possible depth (max 15m, note depth on bottle) so as not to disturb any layers of phytoplankton in the water column Take care not to hit the bottom
Retrieve sample	Replace bung securely in top of tube and pull up. Empty water into the bucket
Fill sample bottles	Lower plastic bottle into bucket leaving a small air space at top. Fill two plastic bottles with sample water Leave one as it is, put four drops of Lugols iodine per 100ml into the other bottle **immediately** and cap securely. Invert gently to mix. Label each bottle clearly with date, site and whether preserved or not.

Collecting Phytoplankton samples using the van Dorn Bottle

Equipment:

van Dorn sampler
Clean bucket -rinse with sea water
Sample bottles
Lugols Iodine - for preserving one of the samples
Polystyrene bins

Method:

Check gear — Ensure rope is attached correctly, with the weight at bottom, and the messenger weight at the top

Set up sampler — Refer to the instructions and diagram on the following page.

How to set up van Dorn Bottle

Lock bottom end cap open

Push spring-loaded plunger down
Insert bottom loop **A** into groove in lower block **D** , securing the loop by guiding plunger hook **C** into hole in lower block, through the loop

Lock top end cap open

Place the top clip **B** onto rope **A**, close to block **D** and clear of knot in loop **A** to avoid the clip catching on the knot in rope **A**.

Clip **B** must be able to release freely when loop **A** is released

Check rope run

Collect sample

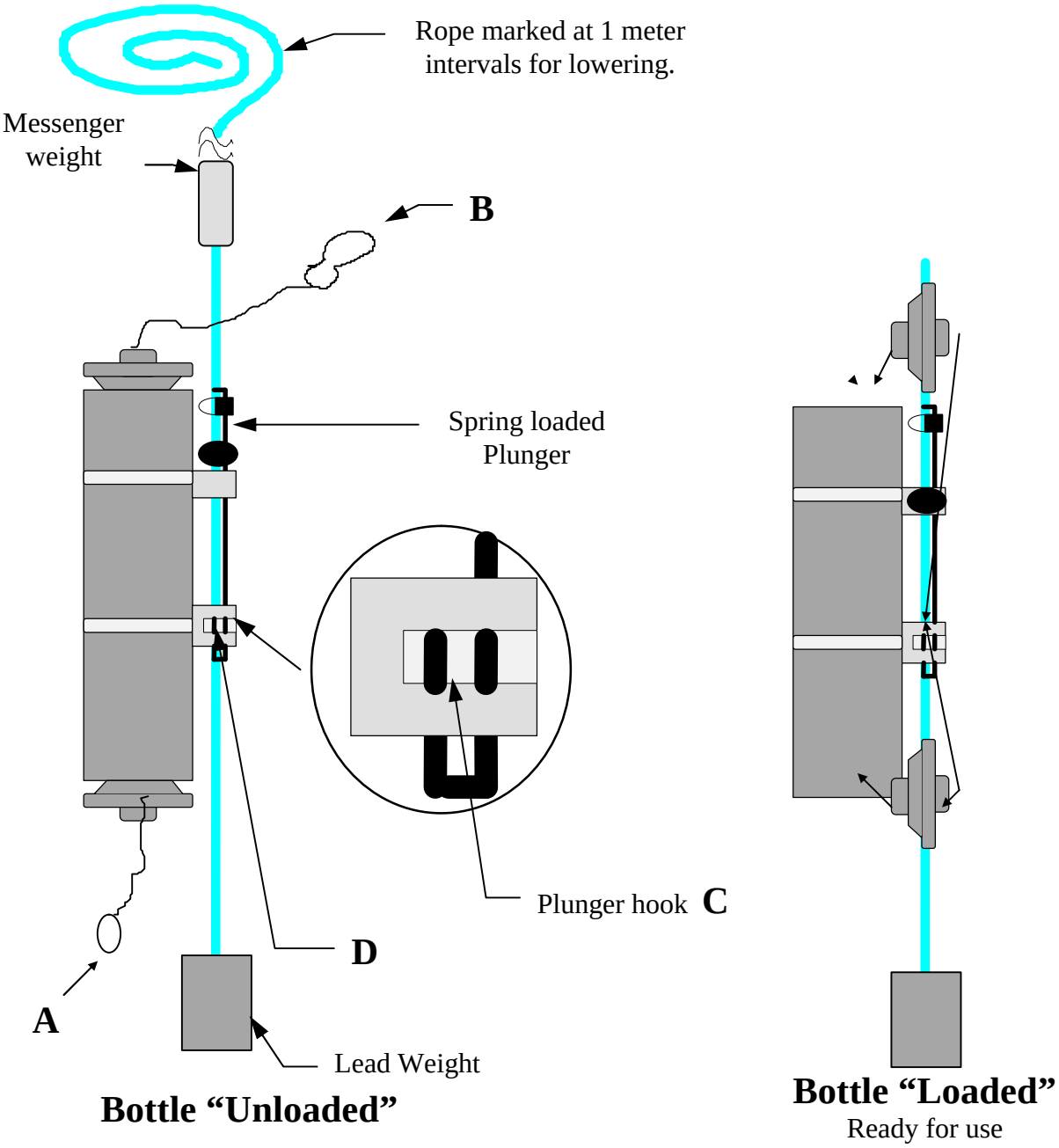
Slowly lower to the desired depth, reading the depth from the rope
Drop messenger weight to close end caps
You will feel through the rope when the end caps have been triggered. A jerk will often trigger stubborn end caps.

Retrieve sample

Haul sampler aboard
Rinse bucket with small quantity of sample
Empty remainder of water into bucket

Fill sample bottles

Lower plastic bottle into bucket leaving a small air space at top
Fill two plastic bottles with sample water
Leave one as it is, put four drops of Lugols iodine per 100ml into the other bottle **immediately** and cap securely.
Label each bottle clearly with date, site and whether preserved or not.


Rope marked at 1 meter intervals for lowering.
Messenger weight
B
Spring loaded Plunger
Plunger hook C
D
A
Lead Weight
Bottle "Unloaded"
Bottle "Loaded"
Ready for use

Collecting phytoplankton using the plankton net

Equipment:

Plankton net (20μm)
Plastic pottle
Lugols iodine

Method

Check gear	Ensure weight is attached to bottom, rope securely tied.
Set up sampler	Place plastic pottle into net, secure with hose clip.
Take sample	Lower to just above the bottom (ideally so the weight doesn't hit and stir up the bottom) **Slowly** but steadily pull the net up.
Wash net	Wash material adhering to inside of net towards the container end by gently dipping and shaking the net.
Preserve sample	Remove sample container and cap. Preserve one sample with four drop's of Lugols Iodine immediately.

Appendix 10 Phytoplankton Species

Category A - Species known to be present in Australian waters and proven to produce toxins either in Australia or internationally:

Alexandrium catenella (saxitoxin and derivatives)
Alexandrium minutum (saxitoxin and derivatives)
Alexandrium ostenfeldii (saxitoxin and derivatives, also produces spirolides in Canada)
Alexandrium tamarense (saxitoxin and derivatives, also has non-toxic strains)
Dinophysis acuminata (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis acuta (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis caudata (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis fortii (pectenotoxin, okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis hastata (okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis mitra (okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis rotundata (okadaic acid?, dinophysis toxins? and diol esters?)
Dinophysis tripos (some strains produce okadaic acid, dinophysis toxins and diol esters)
Gymnodinium catenatum (saxitoxin and derivatives)
Karenia cf *brevis* (brevetoxins)
Prorocentrum lima (okadaic acid?, dinophysis toxins? and diol esters?)
Pseudo-nitzschia australis (domoic acid)
Pseudo-nitzschia delicatissima (domoic acid)
Pseudo-nitzschia fraudulenta (domoic acid)
Pseudo-nitzschia multiseries (domoic acid)
Pseudo-nitzschia pseudodelicatissima (domoic acid)
Pseudo-nitzschia pungens (usually non-toxic, but toxic strains produce high concentrations of domoic acid per cell)
Pseudo-nitzschia turgidula (domoic acid)
Pyrodinium bahamense var. *compressum* (in tropical habitats) (saxitoxin and derivatives)

Category B - Potential toxin producing species (ie toxicity untested/unclear) known to be present in Australian coastal waters

Alexandrium pseudogonyaulax (possible STX and derivatives, goniodomin)
Chattonella marina/antiqua (possible brevetoxins)
Fibrocapsa japonica (possible brevetoxins)
Heterosigma akashiwo (possible brevetoxins)
Pseudo-nitzschia cuspidata (possible domoic acid)
Pseudo-nitzschia heimii (possible domoic acid, non-toxic in New Zealand)
Pseudo-nitzschia lineola (possible domoic acid)
Pseudo-nitzschia multistriata (possible domoic acid, non-toxic in New Zealand)
Pseudo-nitzschia subfraudulenta (possible domoic acid)
Pseudo-nitzschia subpacifica (possible domoic acid)

Category C - Other potential toxin producing species world-wide that may be present in Australian waters.

Alexandrium angustitabulatum (possible saxitoxin and derivatives, present in New Zealand waters)
Alexandrium acatenella (possible saxitoxin and derivatives)
Alexandrium cohorticula (possible saxitoxin and derivatives)
Alexandrium fraterculus (possible saxitoxin and derivatives)
Alexandrium fundyense (possible saxitoxin and derivatives)
Alexandrium lusitanicum (possible saxitoxin and derivatives)
Alexandrium tamiyavanichi (possible saxitoxin and derivatives)
Coolia monotis (produces cooliatoxin)
Dinophysis norvegica (Major DSP producer in Europe)
Gymnodinium aureolum (possible brevetoxins)
Gymnodinium impudicum (possible brevetoxins)
Gymnodinium pulchellum (possible brevetoxins)
Karenia bidigitata (possible brevetoxins, found in New Zealand waters)
Karenia mikimotoi (possible brevetoxins)
Karenia papilionacea (possible brevetoxins)
Karenia selliformis (gymnodimine, found in New Zealand waters)
Karlodinium micrum (possible brevetoxins)
Lingulodinium polyedra (yessotoxin producer in Japan)
Nitzschia navis-varingica (domoic acid, recently confirmed for an isolate from brackish Vietnamese waters)
Ostreopsis siamensis (produces ostreocin)
Pfiesteria piscicida (toxin being characterised)
Prorocentrum concavum (okadaic acid?, dinophysis toxins? and diol esters?)
Prorocentrum elegans (okadaic acid?, dinophysis toxins? and diol esters?)
Prorocentrum hoffmannianum (okadaic acid?, dinophysis toxins? and diol esters?)
Prorocentrum maculosum (produces prorocentrolides)
Prorocentrum minimum (The toxin linked to this organism (185 fatalities in Japan) has not yet been elucidated, and the role of *P. minimum* is still in question)
Protoceratium reticulatum (yessotoxin producer in New Zealand)

(? Indicates this toxin has not been confirmed at the time of this report as being produced by Australian strains of this species)

Appendix 11 Toxic Shellfish Poisoning Case Definitions

Surveillance Case Definition for all Forms of Toxic Shellfish Poisoning

Suspected case (general clinical case definition)

- Vomiting or diarrhoea occurring within 24 hours of consuming shellfish;

- or any of the following neurological symptoms occurring within 24 hours of consuming shellfish:
  - neurosensory
  - paraesthesia, i.e. numbness or tingling around the mouth, face or extremities
  - alternation of temperature sensations such as a prickly feeling on the skin during a bath/shower or exposure to sun, or difficulty distinguishing hot or cold objects
  - neuromotor/neurocerebellar:
    - weakness such as trouble rising from seat or bed
    - difficulty swallowing
    - difficulty breathing
    - paralysis
    - clumsiness
    - unsteady walking
    - dizziness/vertigo
    - slurred/unclear speech
    - double vision;

- or one or more of the following neurological signs/symptoms occurring within 48 hours of consuming shellfish:
  - confusion
  - memory loss
  - disorientation
  - seizure
  - coma

Paralytic Shellfish Poisoning (PSP) Case Definition

Suspected case (clinical case definition)

The following neurological symptoms occurring within 12 hours of consuming shellfish:

- neurosensory;

- paraesthesia, i.e. numbness or tingling around the mouth, face or extremities;
- and one of the following neuromotor/neurocerebellar symptoms:
  - weakness such as trouble rising from seat or bed
  - difficulty in swallowing
  - difficulty in breathing
  - paralysis
  - clumsiness
  - unsteady walking
  - dizziness/vertigo
  - slurred/unclear speech
  - double vision

Probable case

- meets the case definition;

- and detection of PSP biotoxins at or above the regulatory limit in shellfish obtained from near or at the same site (not leftovers) within 7 days of collection of shellfish consumed by the case (current level: 80 µg/100 g shellfish).

Confirmed case

- meets the clinical case definition;

- and detection of PSP biotoxins in leftover shellfish at a level that meant the case consumed a dose likely to cause illness (current level: 10 MU/kg body weight; about 2 µg/kg body weight).

Neurotoxic Shellfish Poisoning (NSP) Case Definition

Suspected case (clinical case definition)

Two or more of the following neurological symptoms occurring within 24 hours of consuming shellfish:

- neurosensory:
  - paraesthesia, i.e. numbness or tingling around the mouth, face or extremities
  - alternation of temperature sensations such as a prickly feeling on the skin during a bath/shower or exposure to sun, or difficulty distinguishing hot or cold objects

- neuromotor/neurocerebellar:
  - weakness such as trouble rising from seat or bed
  - difficulty in swallowing
  - difficulty in breathing
  - paralysis
  - clumsiness
  - unsteady walking
  - dizziness/vertigo
  - slurred/unclear speech
  - double vision

Probable case

- Meets the clinical case definition;

  - and detection of NSP biotoxin at or above the regulatory limit in shellfish obtained from near or at the same site (not leftovers) within 7 days of collection of shellfish consumed by the case (current level: 20 MU/100 g shellfish).

Confirmed case

- meets the clinical case definition;

- and detection of NSP biotoxins in leftover shellfish at a level resulting in the case consuming a dose likely to cause illness (current level: 0.3 MU/kg body weight).

Amnesic Shellfish Poisoning (ASP) Case Definition

Suspected case (clinical case definition)

- Vomiting or diarrhoea or abdominal cramps, occurring within 24 hours of consuming shellfish;

- and no other probable cause identified by microbiological examination of a faecal specimen from the case or microbiological testing of left-over food;

- and/or one or more of the following neurological signs/symptoms occurring within 48 hours of consuming shellfish:
  - confusion
  - memory loss
  - disorientation
  - seizure
  - coma

Probable case

- Meets the clinical case definition;

  - and detection of ASP biotoxin at or above the regulatory limit in shellfish obtained from near or at the same site (not leftovers) within 7 days of collection of shellfish consumed by the case (current level: 20 ppm domoic acid/100 g shellfish).

Confirmed case

- meets the clinical case definition;

- and detection of ASP biotoxins in leftover shellfish at a level resulting in the case consuming a dose likely to cause illness (current level: 0.05 mg/kg body weight).

Diarrhetic Shellfish Poisoning (DSP) Case Definition

Suspected case (clinical case definition)

- Vomiting or diarrhoea occurring within 24 hours of consuming shellfish;
- and no other probable cause identified by microbiological examination of a faecal specimen from the case or microbiological testing of left-over food.

Probable case

- Meets the clinical case definition;
- and detection of DSP biotoxin at or above the regulatory limit in shellfish obtained from near or at the same site (not leftovers) within 7 days of collection of shellfish consumed by the case (current level: 20 µg/100 g shellfish or 5 MU/100 g).

Confirmed case

- meets the clinical case definition;
- and detection of DSP biotoxins in leftover shellfish at a level resulting in the case consuming a dose likely to cause illness (current level: ingestion of 48 µg or 12 MU).

Appendix 12 Phytoplankton Action Levels

The following table summarises the phytoplankton levels (in cells/litre) which are used to trigger sampling of shellfish flesh. The levels relate to discrete or composite samples. These levels are a combination of levels used internationally and in various States in Australia. They should be revised as further monitoring and research is undertaken and supports a change.

Phytoplankton Species	Toxin	Trigger flesh sampling # (Cells per litre)	Issue public health warning (cells per litre)
Alexandrium minutum	PSP	100	5000
Alexandrium ostenfeldii	PSP	100	5000
Alexandrium catenella	PSP	100	5000
Alexandrium tamarense	PSP	100	5000
Alexandrium spp.	PSP?		
Gymnodinium catenatum	PSP	100	5000
Pseudo-nitzschia spp. (>50% total phytoplankton)	ASP	50,000	N/A
Pseudo-nitzschia spp. (<50% total phytoplankton)	ASP	100,000	N/A
Karenia cf *brevis*	NSP	1000	5000
Dinophysis acuminata	DSP	1000	N/A
Dinophysis acuta	DSP	500	N/A
Dinophysis caudata	DSP	500	N/A
Dinophysis fortii	DSP	500	N/A
Dinophysis hastata	DSP	500	N/A
Dinophysis mitra	DSP	500	N/A
Dinophysis rotundata	DSP	500	N/A
Dinophysis tripos	DSP	500	N/A
Total *Dinophysis* spp.	DSP	500	N/A
Prorocentrum lima	DSP	500	N/A

N/A = not applicable.

Note: For *Pseudo-nitzschia spp* risk remains high for a minimum of two weeks post bloom crash.
The trigger levels for the *Alexandrium* species and *Gymnodinium catenatum* have been set at the detection limit of the method used in New Zealand, in order to ensure that detection triggers some action. Different methods and sample volumes may be used by laboratories, and as such these levels should be set at a conservative level to ensure action is taken on detection of cells.

Appendix 13 Marine Biotoxin Regulatory Closure Levels

The following are the regulatory limits for marine biotoxins in the edible portions of shellfish

Paralytic Shellfish Poisoning (PSP)

PSP toxins greater than or equal to 80 µg of saxitoxin equivalent/100 g of edible shellfish flesh (≈ 400 mouse units), by mouse bioassay with a maximum observation time of 1 hour.

Neurotoxic Shellfish Poisoning (NSP)

NSP toxins greater than or equal to 20 mouse units/100 g of edible shellfish flesh, by ether extraction and mouse bioassay with a maximum observation time of 6 hours.

Amnesic Shellfish Poisoning (ASP)

Greater than or equal to 20 ppm (µg/g) of domoic acid in the edible shellfish flesh by high performance liquid chromatography (HPLC).

Diarrhetic Shellfish Poisoning (DSP)

Greater than or equal to 20 µg/100 g of edible shellfish flesh (≈5 mouse units) by 24-hour mouse bioassay or HPLC Electrospray Mass Spectrometry.

NB: DSP toxins include okadaic acid, DTX1, DTX2, DTX3, PTX, PTX2sa, YTX, 45-OH YTX and azaspiracids. There is debate about the human toxicity of some of these compounds, but these should be regulated for as DSP toxins until further testing, including toxicology studies, have been completed and more appropriate levels are able to be set. Internationally this is the accepted way to deal with these toxins where little is known about them.

In November 2001, new EC guidelines were implemented for DSP toxins (G/SPS/N/EEC/141, SANCO/2227/2001 Rev3, Commission of the European Communities), these are as follows:

1) The regulatory limit for total content of Okadaic acid, Dinophysistoxins and Pectenotoxins is fixed at 16 µg/100 g.
2) The regulatory limit for Yessotoxins is fixed at 100 µg of yessotoxin equivalent/100 g.
3) The regulatory limit for Azaspiracids is fixed at 16 µg of azaspiracid equivalents/100 g.

Detection methods for these toxins may be by biological methods – mouse bioassay, rat bioassay. Alternative methods such as HPLC with fluorimetric detection, LCMS, immunoassays and functional assays such as phosphatase inhibition assay may be used as an alternative or complementary to the biological methods, providing that either alone or combined they can detect at least the following analogues:

- Okadaic acid and Dinophysistoxins, with an hydrolysis step possibly required to detect DTX3
- Pectenotoxins: PTX1 and PTX2
- Yessotoxins: YTX, 45 OH YTX, Homo YTX and 45 OH Homo YTX
- Azaspiracids: AZA1, AZA2 and AZA3

Appendix 14 Questionnaire for Case Investigation of Human Illness Following Consumption of Shellfish or Seafood

Insert a standard epidemiological investigation form

Cawthron Institute
Nelson, New Zealand
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