Ecowise Environmental

## Victorian Fisheries Authority

# VICTORIAN MARINE BIOTOXIN MANAGEMENT PLAN
# Version 7.6

## December 2025

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# TABLE OF CONTENTS

**List of Tables**



**Appendices**

# Acknowledgements

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The project team would like to thank the following people for their valuable input which facilitated the completion of this project:

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# 1 Amendments

## 1.1 Amendments

Amendments can be made to this plan by contacting the co-ordinator with the suggested changes and reasons for them.

Industry is responsible for the management of their seafood safety risks under the Seafood Safety Act 2003 and the Food Standards Code for seafood and is consequently responsible for any amendment to this plan. Industry should only amend the plan where the amendment to be made is consistent with an amendment to the ASQAP Manual and the relevant legislation and is supported by relevant advice provided by a food safety expert and other relevant scientists.

To become part of this plan, amendments need to be issued with a covering letter. Amendments are identified by the issue number in the page header, by a vertical line in the left margin adjacent to the line(s) that has been changed and, in the amendments, record on page i. Amendments will be numbered in sequence.

## 1.2 Amendment Record

It is important this plan is kept up to date by the prompt incorporation of amendments and recording in the amendment table on page i.

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.

# 2 Acronyms and Glossary of Terms

## 2.1 Acronyms

| | |
|---|---|
| ASP | Amnesic Shellfish Poisoning (Toxin: domoic acid) |
| ASQAAC | Australian Shellfish Quality Assurance Advisory Committee |
| ASQAP | Australian Shellfish Quality Assurance Program |
| AZA | Azaspiracid |
| AZP | Azaspiracid Shellfish Poisoning (Toxins: AZA-1. AZA-2, AZA-3) |
| BTX | Brevetoxins |
| C toxins | Di-sulphated saxitoxin analogues |
| CEC | Commission of European Communities |
| DAFF | Department of Agriculture, Fisheries and Forestry |
| DH | Department of Health, Victoria |
| DEDJTR | Department of Economic Development, Jobs, Transport and Resources, Victoria |
| DSP | Diarrhetic Shellfish Poisoning (Poisons – OA, DTX 1-3, PTX ) |
| DTX | Dinophysistoxin |
| EE or Ecowise | Ecowise Environmental |
| ELISA | Enzyme Linked Immuno-Sorbent Assay |
| EPA | Environment Protection Agency, Victoria |
| FSANZ | Food Standards Australia & New Zealand |
| FSC | Food Standards Code |
| GTX | Gonyautoxins |
| HPLC | High Performance Liquid Chromatography |
| LCMS/MS | Liquid Chromatography – Mass Spectrometry/Mass Spectrometry |
| MAFRI | Marine & Freshwater Resources Institute |
| VMBMP | Victorian Marine Biotoxin Management Plan |
| MS | Mass spectrometry |
| MU | Mouse Units |
| NATA | National Association of Testing Authorities |
| neoSTX | Neosaxitoxin |
| NSP | Neurotoxic Shellfish Poisoning (Toxins: BTX) |
| OA | Okadaic acid |
| PPB | Port Phillip Bay |
| PSP | Paralytic Shellfish Poisoning (Toxins: STX, GTX, neoSTX, C toxins etc) |
| PTX | Pectenotoxins |
| PTX-2-SA | Pectenotoxin-2-seco acids |
| SSCA | State Shellfish Controlling Agency |

| | |
|---|---|
| STX | Saxitoxins |
| TSP | Toxic Shellfish Poisoning |
| VMBMP | Victorian Marine Biotoxin Management Plan |
| VSOM | Victorian Shellfish Operations Manual |
| VSQAP | Victorian Shellfish Quality Assurance program |
| WES | WATER ECOscience |
| WP | Western Port |
| YTX | Yessotoxins |
| mg/kg | Milligrams per kilogram |
| ug/100g | Micro-grams per 100 grams |

## 2.2 Glossary of Terms

| | |
|---|---|
| Authorised Officer | An officer authorised under the relevant legislation. |
| Growing Area | A marine or enclosed body of water (for example: bay, harbour, gulf, cove, lagoon, inlet, estuary or river) in which commercial species of bivalve molluscs grow naturally or are grown by means of aquaculture. |
| Harvesting Area | An area that has been designated by the Authority for the purpose of growing and harvesting commercial quantities of shellstock for human consumption and may include wild stock or aquaculture shellstock. |
| Authority | The government entity having the legal authority to implement the Food Standards Code - Standard 4.2.1 Primary Production and Processing Standard for Seafood in Victoria. |
| Victorian Fisheries Authority | Means either Victorian Fisheries Authority as an Authority of the State of Victoria or as a division of the Department of Jobs, Skills, Industry and Regions. It was, previously a division of the Department of Economic Development, Jobs, Transport and Resources and before that the Department of Environment, and Primary Industries. |

# 3 Introduction

## 3.1 Background

Some species of marine microalgae (phytoplankton) produce natural toxins which may accumulate in the tissues of filter feeding shellfish. Toxic shellfish poisoning (TSP) may result in humans that have consumed contaminated shellfish.

Within Victoria, four shellfish poisoning syndromes are potentially of concern:

- Paralytic Shellfish Poisoning (PSP)
- Amnesic Shellfish Poisoning (ASP)
- Diarrhetic Shellfish Poisoning (DSP)

Other possible TSPs that have not been detected in Victorian shellfish to date include:

- Neurotoxic Shellfish Poisoning (NSP)
- Azaspiracid Poisoning (AZP)
- Yessotoxins

The potentially causative organisms of these poisoning syndromes are provided in Sections 8.6 and 8.7.

The presence of biotoxins in shellfish not only poses a health risk to consumers but may also adversely impact on the aquaculture industry by lowering consumer confidence in the harvested shellfish product. These risks can be managed by the Victorian Marine Biotoxin Management Plan (VMBMP).

The second edition of the Victorian Marine Biotoxin Management Plan was developed for the Victorian Shellfish Quality Assurance Program (VSQAP) by Ecowise Environmental (EE) in conjunction with the Department of Primary Industries (DPI), Fisheries Victoria.

A new shellfish quality assurance program, the Victorian Shellfish Operations Manual (VSOM) was developed in 2009. This third edition of the Victorian Marine Biotoxin Management Plan reflects the second edition and the changes resulting from the implementation of the VSOM. Ecowise Environmental was engaged by Fisheries Victoria to review the technical aspects of the VMBMP and to incorporate material provide by Fisheries Victoria relating to administration, Harvest Area closure and reopening, agency responsibilities and contacts relating to the inception of the VSOM.

The fourth edition was updated by the Department of Economic Development, Jobs, Transport and Resources with assistance from Dr Steven Brett of Microalgal Services Pty Ltd.

The fifth edition is updated by the Victorian Fisheries Authority to align with the revised VSQAP.

### 3.1.1 History of Biotoxin Surveillance

A history of Victorian shellfish quality assurance phytoplankton and biotoxin surveillance is presented in Section 5.7 of the *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming* (Todd, 2001).

In summary, the Victorian Shellfish Quality Assurance Program was established in 1987 to provide for the safe harvest of blue mussels commercially harvested for the purpose of human consumption. At that time, it serviced four aquaculture zones in Port Phillip Bay (PPB) (Clifton Springs, Grassy Point, Dromana and Beaumaris) and another in Western Port (WP) (Flinders Bight).

Wild stock mussels from the Gippsland Lakes and scallops from PPB and Bass Strait have also been included in the VSQAP program in the past. The program was operated by the Marine and Freshwater Resources Institute (MAFRI) for Fisheries Victoria and was funded entirely by the latter until it was discontinued at the end of 1996. The program collected surface water samples and tissue samples on a regular basis, analysing the samples for phytoplankton and biotoxin respectively. This sampling regime provided for the monitoring of toxic phytoplankton species and biotoxins in commercial shellfish harvest areas.

During the absence of a formal Government run shellfish quality assurance monitoring program in Victoria from July 1997 until August 1999, WES (now Ecowise) performed phytoplankton monitoring for the mussel industry, either as the Victorian Mussel Growers Association or as individual growers, contracted WES to conduct phytoplankton and biotoxin monitoring.

Ecowise Environmental performed all of the subsequent monitoring and reporting components of the Victorian Shellfish Quality Assurance Program (VSQAP), and prepared and reviewed the Victorian Marine Biotoxin Management Plan, under contract to Fisheries Victoria.

From 1990 till 2003 under the VSQAP, phytoplankton and tissue PSP testing has been performed fortnightly at each of the five harvesting areas in PPB and WP, except Beaumaris. Shellfish harvesting at Beaumaris, and consequently monitoring, ceased in March 2001 and this area is no longer authorised for the harvest of bivalve shellfish for human consumption. ASP testing has also been performed each fortnight at the Clifton Springs and Flinders harvesting areas. From 2004 until 2009, the frequency of routine biotoxin testing was reduced to monthly. As a result, the VSQAP has provided a significant database to support decisions in regard to biotoxin management and risk.

In order to classify two additional areas of water within PPB, monitoring has been performed at the Pinnace Channel harvesting area, located in central PPB, since December 2003 and the Mount Martha harvesting area, located in eastern PPB, since August 2006.

The Pinnace Channel harvesting area was formally incorporated into the VSQAP in July 2007 and its initial comprehensive sanitary survey was completed in 2009. The Mount Martha harvesting area was formally incorporated into the VSQAP in January 2008 and its initial comprehensive sanitary survey was completed in 2009.

In 2009 shellfish quality assurance in Victoria was transitioned to an industry managed program described in the Victorian Shellfish Operations Manual (VSOM) oversighted and regulated by Government. At that time the VSQAP monitoring program was reviewed and risk assessed resulting in a number of improvements in both safety and cost. Amongst a number of changes, fortnightly phytoplankton sampling was introduced to act as an early warning trigger for shellfish tissue biotoxin sampling.

Since 2014, two shellfish wild fisheries have been developed in Victoria: the pipi fishery at Discovery Bay, and the scallop dive fishery in Port Phillip Bay. The scallop fishery includes two harvest areas: the Pinnace Channel Scallop Harvest Area and the North-west Port Phillip Scallop Harvest Area. Marine biotoxin monitoring plans for these areas have been developed taking into account the available historic biotoxin data for the area and shellfish species, the

environmental conditions at the harvest areas, and the requirement for data to support planned modification of the sampling plans in the future. These plans are documented in the on-going management plans associated with each harvest area and approved by PrimeSafe. They incorporate fortnightly shellfish sampling of pipis and scallops, combined in the case of scallops with phytoplankton sampling in alternate weeks.

Both scallop harvest areas ceased monitoring in 2022 and are no longer classified or approved for Harvest for human consumption, though scallops monitoring is occurring within the Pinnace Channel Harvesting area, which is a sub area of the original Pinnace Channel Scallop Harvest area.

## 3.2 Aims and Objectives

The principal aim of the Victorian Marine Biotoxin Management Plan is to provide for the protection of shellfish consumers from the hazards of marine toxic shellfish poisoning (TSP) from the commercial harvesting of bivalve shellfish for human consumption from shellfish harvesting areas within PPB and WP in Victoria.

The following objectives have been established to meet this aim:

- The maintenance of a monitoring program using phytoplankton monitoring in conjunction with biotoxin testing of bivalve shellfish tissue. Phytoplankton monitoring is used to provide early warning of the presence of phytoplankton with the potential to contaminate shellfish with marine biotoxins. The results of this monitoring may be used to initiate biotoxin testing, and in some cases harvesting closures. Shellfish tissue biotoxin levels are used to make harvesting reopening and regulatory decisions.
- To document all procedures and contacts required to effectively manage incidents of shellfish biotoxin contamination.
- To facilitate the harvesting of shellfish which are free from marine biotoxins.
- To provide an effective and co-ordinated response to marine biotoxin events, minimising the risk of human illness.
- Ensure public awareness of shellfish biotoxin events while minimising potential adverse publicity to the shellfish industry.
- Maintain updated management protocols (contingency plans) to allow rapid and effective responses to marine biotoxin events.

## 3.3 Scope

The Victorian Marine Biotoxin Management Plan is designed primarily for the commercial aquaculture harvesting of bivalve shellfish from PPB and WP, areas for which extensive phytoplankton records exist. With some modifications, the VMBMP may be adopted for commercial wild shellfish harvesting if appropriate. There is evidence that various shellfish species may not bioconcentrate and metabolise particular biotoxins in the same manner. Hence, some review of biotoxin monitoring protocols may be required should additional shellfish species be grown and commercially harvested within PPB and WP.

## 3.4 Review

This Victorian Marine Biotoxin Management Plan will be reviewed as required to reflect changes to scientific and technical knowledge and the requirements of the Authority. In such cases an updated, numbered "version" will be issued, incorporating all amendments. Reviews shall only be undertaken by Victorian Fisheries Authority staff with good knowledge of the Victorian Marine Biotoxin Management Plan, and the VSQAP and its application in Victoria. This document is Edition 7.6 of the Victorian Marine Biotoxin Management Plan.

Upon issue of an updated version of the Victorian Marine Biotoxin Management Plan, all previous versions are to be destroyed or stored in such a manner that superseded documentation will not be available for use.

# 4 Requirements for a Victorian Marine Biotoxin Management Plan

Division 3 of standard 4.2.1 of the Primary production and processing standard for seafood requires Harvesting areas be subject to a Victorian Marine Biotoxin Management Plan prepared in accordance with the ASQAP Manual or other condition recognised by the Authority. The ASQAP Operations Manual (2024), specifications are that a biotoxin management plan must define:

- The responsibilities of all parties involved in the management plan
- Hydrographical details describing predominant currents and circulatory patterns
- Species of shellfish cultured/harvested
- Sample sites
- Sampling frequencies
- Sampling methods
- Methods of analysis for water and shellfish samples
- Laboratories used for sample analysis
- Alert level/s for toxic/potentially toxic algal species
- Potentially toxic algal species list
- Actions to be taken by the Authority when either alert levels are exceeded, or toxins are found in shellfish below closure levels
- Closure procedures including closure criteria, notification of closures to marine farmers and relevant authorities, public announcements, management during closures, product recall
- Opening procedures including opening criteria, notification of opening to marine farmers and relevant authorities, public announcements, procedures for opening inactive or seasonal growing areas
- Case definitions of toxic syndromes

# 5 Administration

## 5.1 Legislation and Guidelines

A list of Federal and State legislation and guidelines that may be relevant to biotoxin management are provided below. For further detail, refer to the relevant document.

### 5.1.1 Federal

#### 5.1.1.1 Legislation

- Food Standards Australia New Zealand Act 1991 and its subordinate Australian New Zealand Food Safety Code (the ANZFSC) and Standard 4.2.1 - Primary Production and Processing Standard (the PPPS)
- Export Control Act 1982 and its subordinate Export Control (Fish & Fish Products) Orders 2005 and the Export Control (Prescribed Goods General) Order 2005

#### 5.1.1.2 Guidelines

- Australian Shellfish Quality Assurance Program (ASQAP) Operations Manual (2024)
- Australian and New Zealand Guidelines for Fresh and Marine Water Quality (2000)

### 5.1.2 State

- Public Health and Wellbeing Act 2008.
- Fisheries Act 1995.
- Food Act 1984.
- Seafood Safety Act 2003.
- Environment Protection Act 2017.

## 5.2 Roles and Responsibilities

### 5.2.1 Victoria Fisheries Authority

The following are the responsibilities of Victoria Fisheries Authority

- Issue licences authorising aquaculture activity and wild take under the Fisheries Act 1995.
- Maintain and revise this MBMP as required.
- Advise on the classification of harvesting areas and the revision of the MBMP and the VSQAP.
- Oversight:
    - industry sampling.
    - opening and closures due to phytoplankton and or biotoxin.
    - preparation of annual reports.
- Oversight comprehensive sanitary surveys and triennial reviews.

- Maintain databases for phytoplankton and biotoxin.
- Provide expert advice to industry.
- Provide representation at the national Australian Shellfish Quality Assurance Advisory Committee (ASQAAC).
- Provide in field observation and reporting of suspected compliance breaches

### 5.2.2 PrimeSafe

PrimeSafe is the authority responsible for administering the Seafood Safety Act (Victoria) 2003. The Seafood Safety Act 2003 requires seafood businesses (includes commercial bivalve shellfish harvesters for human consumption and bivalve shellfish processors) to be licensed and to have in place an approved seafood safety plan.

PrimeSafe's functions include:

- Control and review standards for construction and hygiene at seafood processing facilities.
- Licence seafood businesses including processing premises, harvesting vessels and vehicles handling seafood.
- Inspection of systems and audited quality assurance programs.
- Enforcement of necessary sanitary controls for processing plants and vehicles handling seafood.
- Detain and recall product considered unfit for human consumption.
- Regulate the processing of shellfish for human consumption by licensing, approval of food safety plans and auditing of compliance
- Implement the Food Standards Code for seafood in Victoria

### 5.2.3 Department of Health (Victoria)

The following are the responsibilities of the Department of Health (DH).

- Detain and recall product considered unfit for human consumption.
- Provide expert advice to the Authority.
- Licence food transport vehicles (subject to the Seafood Safety Act 2003).
- Maintain epidemiological data for notifiable diseases (including TSP cases).

### 5.2.4 Local Government

The following are responsibilities of Local government through the Food Act 1984.

- Licence relevant businesses to handle seafood (subject to the Seafood Safety Act 2003) (for example: supermarkets).
- Enforce necessary sanitary controls for processing plants and vehicles handling seafood.

- Provide advice concerning local sewage spills/events.

### 5.2.5 Department of Agriculture, Fisheries and Forestry (DAFF)

DAFF is the Commonwealth 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.

DAFF administers the export inspection program, which includes provision for:

- The registration of premises, including vehicles, which prepare shellfish intended for export.
- The inspection of registered export establishments for implementation of good food processing practises.
- Conducting HACCP based food processing controls for exporters.
- Auditing state shellfish quality assurance programs for export accreditation and for compliance with the Export Control Act (Commonwealth) 1982 and its subordinate orders including the *Export Control (Fish & Fish Products) Orders 2005*.

DAFF 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 (Fish & Fish Products) Orders 2005* also regulate the controls for export of shellfish and 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 for shellstock intended for export.

### 5.2.6 Shellfish Harvesting Industry

The following are the responsibilities of the industry who harvest bivalve shellfish for human consumption.

- Comply with the requirements of their PrimeSafe licence.
- Manage their seafood safety risks.
- Ensure no harvesting takes place when a closure is in place.
- Undertake a notification process, when required, for the recall of contaminated shellfish.
- Control the harvesting of shellfish based on sanitary conditions.
- Undertake the sampling program.
- Sub-contract components of the program to the private sector where required.
- Ensure no illegal harvesting takes place when a closure is in place.
- Retain records of closure and re-opening notices for harvesting areas.
- Retain records of monitoring, sampling and harvesting of harvesting areas.
- Provide representation at the national Australian Shellfish Quality Assurance Advisory Committee (ASQAAC).

### 5.2.7 Environment Protection Authority (EPA Victoria)

- Provide expert advice to PrimeSafe, Victoria Fisheries Authority, harvesters and the community concerning events adversely affecting water quality in PPB and WP.

### 5.2.8 Australian Shellfish Quality Assurance Advisory Committee

- Provide guidance on shellfish safety and quality.
- Provide a set of guidelines for states and territories (the ASQAP Operations Manual).
- Be responsible for the formulation and regular updating of the ASQAP Operations Manual.

# 6 Hydrographical Details of Harvesting Areas

This Victorian Marine Biotoxin Management Plan has been prepared in respect of Harvesting Areas in the following geographical locations:

(i) Clifton Springs Harvesting Area

(ii) Dromana Harvesting Area

(iii) Flinders Harvesting Area

(iv) Grassy Point Harvesting Area

(v) Mount Martha Harvesting Area

(vi) Pinnace Channel Harvesting Area

(vii) Pinnace Channel Scallop Harvest Area

(viii) North-west Port Phillip Scallop Harvest Area

(ix) Discovery Bay Pipi Harvest Area.

The hydrographical details describing predominant currents and circulatory patterns are provided in the relevant sections of the management plans and referenced documents that cover each harvesting area:

*Geelong Arm Aquaculture Fisheries Reserves Management Plan (2005):*

- Clifton Springs Harvesting Area
- Grassy Point Harvesting Area

*Eastern Port Phillip Bay Aquaculture Fisheries Reserves Management Plan (2005):*

- Dromana Harvesting Area
- Mount Martha Harvesting Area

*Flinders Aquaculture Fisheries Reserve Management Plan (2005):*

Flinders Harvesting Area

*Pinnace Channel Aquaculture Fisheries Reserve Management Plan (2005):*

- Pinnace Channel Harvesting Area

*Sanitary Survey Report for the Discovery Bay Growing Area*

*Proposal To Extend Classification Of Pinnace Channel Harvest Area To Harvest Of Scallops*

*Proposal For Classification Of Northwest Port Phillip Bay Scallop Harvest Areas*

Detailed material may also be found in the following supporting documents all published as part of the Fisheries Victoria Report Series:

- *Geelong Arm Aquaculture Fisheries Reserves – current, wind and wave data (2004)*
- *Environmental Characterisation of the Aquaculture Fisheries Reserves in the Geelong Arm, Port Phillip Bay, Victoria (2004)*

- *Eastern Port Phillip Bay Aquaculture Fisheries Reserves – current, wind and wave data (2004)*
- *Environmental Characterisation of the Aquaculture Fisheries Reserves in Eastern Port Phillip Bay, Victoria (2004)*
- *Environmental Characterisation of the Flinders Aquaculture Fisheries Reserve in Western Port, Victoria (2004)*
- *Baseline Data for the Pinnace Channel Aquaculture Site (2001)*
- *Pinnace Channel Fisheries Reserve - current, wind and wave data (2003)*
- *Bathymetric Survey of the Proposed Aquaculture Zone, Pinnace Channel Port Phillip (2001)*

# 7 Species of Shellfish Cultured and Harvested

The species of shellfish covered by this Victorian Marine Biotoxin Management Plan are:

- Blue mussels, *Mytilus galloprovincialis,*
- Native oyster, *Ostrea angasi*
- Scallops, *Pectin fumatus*

Pipis, (*Plebidonax deltoids*) at Discovery Bay are covered by the Interim Biotoxin Management Plan for the Discovery Bay

# 8 Monitoring

## 8.1 Monitoring Program Goals

The Victorian Marine Biotoxin Management Plan provides a phytoplankton and biotoxin monitoring program that has been designed with the following goals in mind:

- Provide early warning of potential marine biotoxin contamination by detecting changes in the presence and abundance of potentially toxic phytoplankton species.
- Increase the knowledge and a wider understanding of the presence of those species that pose a potential marine biotoxin threat to commercial harvesters of shellfish for human consumption.
- Establish a long-term data set of phytoplankton abundance, marine biotoxin levels and events, and associated ecological factors. This dataset may be used to improve risk assessment, facilitate the analysis of trends in phytoplankton abundance and aid the prediction of marine biotoxin events.
- Provide toxic phytoplankton abundance trigger levels that permit harvesting closures in a timely manner before biotoxins reach levels that may threaten human health.
- Provide biotoxin levels to permit harvesting area closures and re-openings in a timely and safe manner.
- Validate that phytoplankton monitoring captures all toxic events where the risk assessment requires.
- Maintain an up-to-date list of local, national and international potentially toxic phytoplankton species.

## 8.2 Sampling Sites

When sampling sites for toxic phytoplankton and shellfish are established, the following general factors were considered:

- The history of phytoplankton and marine biotoxin levels in PPB and WP.
- The need to monitor effectively the entirety of all aquaculture shellfish harvesting areas.
- Location of bivalve shellfish being harvested at various times.
- Accessibility of sample sites in various weather conditions.
- Environmental factors likely to influence sampling, such as:
    - Major currents.
    - Retention zones and circular patterns.
    - Areas where algal blooms and fish kills were regularly observed or had been regularly observed in the past.
    - Impact of rivers.
    - Impact of drains.

- Any other factors that may have influenced sampling.

- Sites have been chosen so that the water being sampled for phytoplankton is representative of the water being filtered by the shellfish within the Harvesting Area.
- For line culture, the water samples are collected so that the entire depth of the lines bearing shellfish is sampled, to account for the possibility of uneven vertical distribution of phytoplankton.

The aquaculture harvesting areas within PPB and WP are all in open, well circulated and vertically mixed waters. Shellfish and phytoplankton are sampled where suitable shellfish are available and where harvesting for human consumption is to occur. Consequently, sampling maybe carried out at different sites within each harvesting area over consecutive sampling events.

## 8.3 Sampling Officers and Sample Collection

It is a NATA requirement that sampling be undertaken by appropriately trained personnel. In addition, a suitably trained Victorian Fisheries Authority staff member will be available to undertake training of samplers and the provision of advice when required.

All sampling must be performed in accordance with the sampling protocols provided in this Victorian Marine Biotoxin Management Plan.

Sample collection forms as provided in the VSQAP must be completed with each sample event. These provide a chain of documentation of any observations made within the harvesting areas, such as weather conditions, or anything else that may be relevant to the sample collection process and sample integrity. Industry must retain a copy of the sample collection forms on file. All sample collection forms are made available to PrimeSafe and its nominated auditor upon request.

Where scheduled samples cannot be collected during any sampling event, this is recorded in the auditable documentation and reported to the Authority (site and reason) as soon as possible.

## 8.4 Sampling Safety

It is the responsibility of the sampler and boat master to ensure that all sampling is undertaken in a safe manner that does not endanger human safety and is consistent with all legislative requirements.

## 8.5 Phytoplankton Monitoring

### 8.5.1 Sampling Frequency

Since 1987, phytoplankton sampling has been carried out at all harvesting areas within PPB and WP, usually on a fortnightly basis. Consequently, a considerable body of data exists concerning phytoplankton blooms in these waters.

Phytoplankton sampling is carried out in all harvesting areas on a fortnightly, routine basis during harvesting times, which is normally all year round. The frequency of sampling has been found to be adequate to allow phytoplankton monitoring to provide early warning of the potential

for biotoxin contamination of bivalve shellfish tissue and as a trigger to initiate tissue sampling for biotoxin analysis. The determination of the presence or absence of potentially biotoxin producing phytoplankton in water samples is undertaken consistent with the requirements of the Food Standards Code and the Export Orders.

Where phytoplankton monitoring reveals the presence of potentially toxic phytoplankton species in numbers equal to or above the trigger levels then additional biotoxin testing must be undertaken.

Where phytoplankton monitoring reveals the presence of potentially toxic phytoplankton species in numbers approaching the trigger for biotoxin testing, or in rising numbers, the sampling frequency should be increased to monitor the state of the bloom. Once the bloom has degenerated the sampling frequency may be reduced.

### 8.5.2 Sampling Methods

Detailed instructions for the use of appropriate phytoplankton sampling equipment are presented in Appendix 6.

Two samples, a concentrated plankton net haul and a depth-integrated hosepipe sample, are collected for phytoplankton analysis:

- A concentrated sample using a 6m vertical haul with a 20µm mesh plankton net; this sample is used to identify any potentially toxic or nuisance species present, particularly those with very low abundance trigger levels. Although there is the potential that fragile algae such as the non-armoured gymnodinioids may be damaged by the use of these nets, experience has shown that these cells are sampled intact when the nets are used appropriately.
- The concentrated net sample is collected in a 75mL polycarbonate vial attached to the net, and transferred to a separate, larger storage bottle leaving a 20 – 30 mm air space and capped tightly. The sample is labelled appropriately with the date and time of sampling, sample type and the Harvesting Area.
- A depth-integrated sample is collected using a 6 m long, 25mm internal diameter hosepipe sampler and placed into a clean bucket on board the sampling vessel. This is mixed thoroughly taking care to avoid damage to any phytoplankton present and a 1 L sub-sample collected for enumeration. A 20 – 30 mm air space left prior to capping. The sample is labelled with the date and time of sampling, sample type and the Harvesting Area.
- All samples are collected so that foreign inclusions are avoided (*e.g.* outboard motor oil).
- All samples are stored in an upright position in an esky containing a small ice pack that does not contact the samples – the purpose of the icepack is solely to prevent the interior of the esky from heating up and not to cool the sample(s).
- Excess shaking of the samples during transport and sampling is to be avoided as this may damage some phytoplankton.

### 8.5.3 Laboratory used for phytoplankton analysis

It is a requirement of the VMBMP and the VSQAP that the analysis of all phytoplankton samples be undertaken at NATA registered laboratories or international laboratories with quality

assurance programs of equivalent standard. Appendix 2 lists the name and contact details of organisations that, at the time of writing, may be used to provide analytical services for the identification and enumeration of phytoplankton.

## 8.6 Phytoplankton Species Monitored

Appendix 7 contains lists of phytoplankton species present or likely to be present in Australian waters sorted into the following categories on the basis of their likelihood of occurrence and potential for toxicity:

- Category A1 - Species known to be present in southern Australian waters including PPB and WP, and proven or suspected toxin producers in Australia.
- Category A2 - Species known to be present in Australian waters and proven to produce toxins in Australia or overseas.
- 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 worldwide that may be present in Australian waters.

The phytoplankton monitoring program must at all times be able to identify potentially toxic species on these lists, particularly those in Categories A & B. In some cases, where species identification is difficult, or the taxonomy is unclear, similar species may be managed as a single group. For example, despite the fact that only two species encountered have a record of toxicity, all *Pseudo-nitzschia* spp. are initially assumed to be as toxic as the most toxic member of the group. This allows for conservative management until definitive identification is made. This principle is also applied to any case where the identification of a potentially toxic species is uncertain.

Appendix 9 lists the trigger levels for phytoplankton species within PPB and WP. These relate to enumeration using an integrated phytoplankton sample collected with a hosepipe sampler. These triggers are used to initiate tissue biotoxin testing and precautionary harvesting closures pending biotoxin results.

## 8.7 Tissue biotoxin monitoring

### 8.7.1 Sampling Frequency

*Aquaculture Harvest Areas*

- PSP, DSP and ASP biotoxin tissue analysis is carried out monthly at all Harvest areas or more frequently when potentially toxic phytoplankton abundance levels indicate additional monitoring is necessary. This monitoring commenced in 2017 to meet export requirements.
- From 2009 to 2017 biotoxin analysis was performed only when potentially toxic phytoplankton abundance levels indicated this was necessary. This reduction in biotoxin monitoring followed an independent risk assessment and development of the VSOM.
- Routine PSP biotoxin tissue analysis was carried out from 1999 till 2009,
- Until January 2006, ASP (domoic acid) biotoxin tissue analysis was carried out routinely every four weeks at the Clifton Springs and Flinders harvesting areas, to provide

background data for the two major bays where aquaculture harvesting areas exist. Domoic acid has not been recorded in mussels from any harvesting area in PPB but has been recorded at very low levels in scallops from Port Phillip Bay and only once in mussels from WP at very low levels.

- Other biotoxin analyses (NSP, AZP) are performed when potentially toxic phytoplankton abundance levels indicate this is necessary.

*Scallop Harvest Areas:*

As a minimum, sampling frequency meets the requirements specified for aquaculture areas. Additional scallop sampling is undertaken as per the management plans for each harvest area.

*Pipi Harvest Areas:*

As a minimum, sampling frequency meets the requirements specified for the Discovery Bay pipi area. Currently additional pipi sampling is being undertaken as per the management plan for that harvest area.

### 8.7.2 Shellfish Species Sampled

The shellfish species sampled for marine biotoxin analysis are those that are harvested for human consumption. Currently this includes: Blue mussels, *Mytilus galloprovincialis*; Angasi oysters, *Ostrea angasi*, and Commercial scallop *Pecten fumatus* (Pinnace Channel Harvest Area).

The tissue portions to be analysed must match the product that is to be marketed i.e., whole tissues for mussels, oysters, pipis, and scallops, unless only the scallop muscle tissue and roe are supplied to the market, in which case muscle and roe only are tested.

### 8.7.3 Methods

#### 8.7.3.3 Sampling

- For areas where the risk rating is unknown due to insufficient historical data shellfish are collected routinely for biotoxin analysis.
- For areas where the risk is assessed as low shellfish are collected for tissue biotoxin analysis when phytoplankton samples are approaching or exceeding early warning alert levels at a Harvesting area.
- For each mussel biotoxin analysis, 30 - 40 large mussels are required from each site sampled. For scallops, 25 market-sized scallops are required. (Mussels are used as indicator species for oysters.) Mussels are shucked in the laboratory and 150 – 180 g flesh prepared for each biotoxin analysis.
- Shellfish should be transported after collection in eskies containing ice packs to keep them cool. Shellfish maybe frozen for later analysis.

#### 8.7.3.4 Laboratory Testing

Analytical laboratories undertaking marine biotoxin analysis of shellfish samples must be NATA-accredited (or for overseas laboratories, an equivalent accreditation) for the tests undertaken. Symbio Laboratories in Sydney and Analytical Services Tasmania, both NATA certified laboratories, can perform the full range of biotoxin analyses required by the Food Standards Code which includes PSP, ASP, DSP and NSP, plus analysis for additional toxins such as azaspiracids and yessotoxins if required.

Appendix 3 lists the organisations that can provide analytical services for biotoxin analysis of shellfish tissue samples. Details of the methodologies used are provided in Appendix 4.

There are four main groups of toxins of concern within Australia that may accumulate in shellfish tissue and cause illness in humans. These are named after the poisoning syndrome they cause. The regulatory limits applied within the Victorian Marine Biotoxin Management Plan meet and in some cases are more conservative than those of the FSANZ Food Standards Code (FSC).

##### Paralytic Shellfish Poisons (PSPs)

A range of Paralytic Shellfish Toxins such as STX, C toxins and gonyautoxins are produced by several dinoflagellate species including *Alexandrium catenella, A. minutum, A. tamarense* and *Gymnodinium catenatum*. These toxins may be fatal to human consumers of contaminated shellfish through respiratory paralysis, although this is rare and there have been no fatal cases in Australia. PSP was detected in PPB mussels in 1993 and 1994 at the Clifton Springs and Grassy Point harvesting areas; the most likely source was *A. tamarense* (Arnott *et al*, 1999). The maximum PSP concentration detected was 276 μg/100g at Clifton Springs.

Current testing:

*Testing Agency for PSP: Symbio Laboratories, New South Wales.*

*Method: PST confirmation by ENV132 HPLC-FLD*

*Testing Agency for PSP: Analytical Services Tasmania.*

*Method: PST confirmation by Boundy Method (3416) LC-MS/MS*

*Units: mg/kg (or μg/100g)*

*FSC Regulatory Limit: 0.8 mg/kg (STX Equivalent)*

##### Amnesic Shellfish Poisons (ASPs)

Amnesic shellfish poisoning is caused by domoic acid produced by several species of diatoms belonging to the genus *Pseudo-nitzschia*, such as *P. australis* and *P. multiseries*. ASP may cause symptoms from nausea, vomiting and abdominal cramps to dizziness, hallucinations, short-term memory loss and seizures. Although most species of *Pseudo-nitzschia* are non-toxic, they are very difficult to separate definitively using only light microscopy. Hence, all *Pseudo-*

*nitzschia* are initially assumed to be toxic until definitive identification is made. There are no documented cases of amnesic shellfish poisoning in Australia. Domoic acid has not been detected in Victorian mussels but has been detected in scallops from Bass Strait (Arnott *et al*, 1999).

Current testing:

*Testing Agency for ASP: Symbio Laboratories, New South Wales.*

*Method: ASP confirmation ENV133 by LCMSMS*

*Testing Agency for ASP: Analytical Services Tasmania.*

*Method: ASP confirmation by method (3411) LC-MS/MS*

*Units: mg/kg (or μg/g)*

*FSC Regulatory Limit: 20 mg/kg (Domoic Acid Equivalent)*

### Diarrhetic Shellfish Poisons (DSPs)

A range of DSP toxins such as OA, DTX 1 – 3 and PTX are produced by several species of dinoflagellate including *Dinophysis acuminata, D. acuta, D. fortii* and *Prorocentrum lima*. Diarrhetic shellfish poisons may cause gastrointestinal problems including diarrhoea, vomiting and abdominal pain; recovery occurs within 3 days irrespective of medical treatment (Hallegraeff, 1997). There have been no reported cases of diarrhetic shellfish poisoning within the areas covered by the Victorian Marine Biotoxin Management Plan.

In the past, PTX seco-acids have been included as a DSP toxin. However, subsequent work in New Zealand (MacKenzie 2002) for the Marlborough Sounds Shellfish Quality Program and within Australia (Burgess 2002) has shown that these compounds are not toxic to humans. Consequently, they are no longer regulated as a DSP toxin. Most of the "DSP" found in mussels tested from PPB during *Dinophysis acuminata* blooms, was PTX-2-SA.

DSP toxins are not defined within the ANZFSC, Standard 1.4.1 but, as noted in the ASQAP Operations Manual, are by both the European Union (Directive 2002/225/EC) and New Zealand Specifications for Bivalve Molluscan Shellfish. The following are included:

- Okadaic acid (OA)
- Dinophysis toxins (DTX)
- Pectenotoxins (PTX)

The FSANZ Food Standards Code Regulatory Limit for DSP is 0.16 mg/kg.

Current testing:

*Testing Agency for DSP: Symbio Laboratories, New South Wales.*

*Method: DSP confirmation ENV133 by LCMSMS*

*Testing Agency for DSP: Analytical Services Tasmania.*

*Method: DSP confirmation by method (3411) LC-MS/MS*

*Units: mg/kg (or μg/100g)*

*FSC Regulatory Limit: 0.16 mg/kg (Okadaic Acid Equivalent) (Total of all DSP toxins).*

### Neurotoxic Shellfish Poisons (NSPs)

Neurotoxic shellfish poisoning is caused by brevetoxins produced by some dinoflagellates, particularly *Karenia brevis.* NSP symptoms vary from headaches, diarrhoea, muscle and joint pain, and vomiting in mild cases, to paraesthesia, altered perception of hot and cold and breathing and swallowing difficulties in extreme cases. Which species produce BTX (brevetoxins) at levels sufficient to cause human intoxication is confounded somewhat by a lack of knowledge of the taxonomy of this group.

The only suspected NSP incident in Victoria was reported in 1994 and resulted from the consumption of wild stock mussels from the Tamboon Inlet in Gippsland. *K.* cf *brevis* was identified as the organism responsible (Arnott, 1998).

In 2025, a large harmful algal bloom affected parts South Australia with NSP brevetoxins subsequently identified within shellfish. *K.brevis* was not present in the bloom and it is suspected the primary causative species was *Karenia cristata,* a novel species in Australia.

Current testing:

*Testing Agency for NSP: Symbio Laboratories, New South Wales.*

*Method: LC-MS/MS*

*Testing Agency for NSP: Analytical Services Tasmania.*

*Method: LC-MS/MS*

*Units: mg/kg*

*FSC Regulatory Limit: 200 MU/kg or 0.8 mg/kg BTX-2 eq*

## Other toxins

### Yessotoxins (YTXs)

YTXs and their derivatives have a structure similar to that of brevetoxins but do not have the same neurological effects. YTXs and their analogues appear to be produced by a number of dinoflagellates including *Protoceratium reticulatum* and *Coolia monotis* (Hallegraeff, 2002). YTX was detected in PPB in August 2011 associated with the algae *Dinophysis acuminata* with maximum levels of 0.027 -0.035 mg/kg. *P. reticulatum* has been found in most Harvesting Areas in Port Phillip Bay since 2011.

YTX is not regulated in Australia through the Food Standards Code and although it is toxic to mice when applied intraperitoneally, its oral toxicity is questionable (Cawthron Institute, 2001). The 32nd Session of the CODEX Committee on Fish and Fishery Products (1-5 October 2012)

confirmed the exclusion of yessotoxins from the list of marine biotoxins that should be tested at international level. However, on 16 Aug 2013 the European Commission's European Food Safety Authority (EFSA) adopted an Opinion of the Scientific Panel to increase the limit to 3.75mg/kg. (Official Journal of the European Union, COMMISSION REGULATION (EU) No 786/2013).

Testing facilities for yessotoxin in shellfish are available.

*Testing Agency for yessotoxins: Symbio Laboratories, New South Wales.*

*Method: LC-MS/MS*

*Testing Agency for yessotoxins: Analytical Services Tasmania.*

*Method: LC-MS/MS*

*Units: mg/kg* (or *µg/100g*)

*Regulatory Limit: Not regulated in Australia, maximum limit applied in Victoria Marine Biotoxin Plan = 3.75 mg/kg.*

### Azapiracids (AZA)

Azaspiracid Shellfish Poisoning (AZP) is caused by a group of toxins with a novel chemical structure, called azaspiracids. AZP has occurred in Ireland and the symptoms include nausea, vomiting, diarrhoea and stomach cramps. The causative agent appears to be some strains of the dinoflagellate *Protoperidinium crassipes* (Hallegraeff, 2002). AZPs have not been detected in Australia or New Zealand.

AZA is not regulated in Australia through the Food Standards Code. The European Guidelines recommended a limit of 16 µg/100g for AZA equivalents.

*Testing Agency for AZA: Symbio Laboratories, New South Wales.*

*Method: LC-MS/MS*

*Testing Agency for AZA: Analytical Services Tasmania.*

*Method: LC-MS/MS*

*Units: mg/kg (or µg/100g)*

*Regulatory Limit: Not regulated in Australia, maximum limit applied in Victoria Marine Biotoxin Plan = 0.16 mg/kg.*

## 8.8 Environmental Information

At the same time as phytoplankton/biotoxin sampling is carried out, salinity, water temperature and the occurrence of rainfall local to the Harvesting Area are also recorded.

## 8.9 Reporting and Notification

- Results from the phytoplankton analyses are provided to the relevant Harvest Area Coordinator (HAC) and shellfish farmers and Victorian Fisheries Authority within 24 hours of receipt. If analytical results reveal the presence of toxic phytoplankton species in significant numbers, the relevant HAC and shellfish farmers must be informed immediately via phone and e-mail.
- Biotoxin results are emailed to the relevant HAC, Victorian Fisheries Authority and shellfish farmers when analysis is complete (2 - 4 days depending on the analysis required and day of sampling).
- If biotoxins are detected above the limit, the laboratory concerned notifies the HAC and Victoria Fisheries Authority immediately. The HAC then immediately notifies the relevant shellfish farmers (or their delegate) by phone and e-mail to inform them of the result, allowing appropriate management action to be taken promptly.
- Should biotoxins be detected in shellfish tissue, it is the responsibility of the appropriate HAC to notify the relevant shellfish farmers (PrimeSafe licence holders), Victorian Fisheries Authority, PrimeSafe and other relevant industry personnel and stakeholders (see Appendix 1). This should be done immediately by telephone and written confirmation provided by e-mail as soon as practicable.
- The approximate schedule for receiving laboratory results is displayed in Table 2.

**Table 1: Approximate schedule for receiving routine sampling results**

| Results | Days | Methods |
|---|---|---|
| Sampling | 0 | |
| Phytoplankton Identification/enumeration | 1 | E-mail |
| DSP, ASP, PSP, NSP, AZP Analyses | 3-8 | E-Mail |

- All reports issued contain comments explaining the significance of any "positive" results obtained and recommend management actions where appropriate.

The relevant authority contacts are presented in Appendix 1.

## 8.10 Data Storage

- Electronic and hardcopy reports of all analytical results must be maintained (stored) in a secure location, by the Harvest Area Coordinator, Victorian Fisheries Authority and PrimeSafe licence holders on their data storage and filing systems, together with a copy of the field sampling sheet.

- Once all analytical results relating to a sampling event are received, the data are to be stored permanently on the Harvest Area Coordinators', Victorian Fisheries Authority and PrimeSafe licence holders' databases.
- The Victorian Fisheries Authority Biotoxin database has been maintained by the Victorian Fisheries Authority since the inception of the VSQAP in 1987 and contains all monitoring data from that date until the end of August 2024. The Harvest Area Coordinators and PrimeSafe licence holders are to retain all biotoxin data. Victorian Fisheries Authority continues to maintain the database for all Harvesting Areas.

## 8.11 Contingency Plans for Marine Biotoxin Events

Contingency plans (management protocols) for each of the known nuisance/toxic species encountered or likely to be encountered in PPB or WP have been formulated. These are attached in Appendix 11.

Each protocol contains the following:

- Title noting phytoplankton species to which it refers.
- Background information concerning the phytoplankton concerned, including toxicity.
- Rationale for the protocol.
- Step by step contingency plan.
- Details of the relevant abundance triggers for tissue testing and harvest suspension.
- Details of the regulatory limits for the relevant toxins.

Management protocols have been prepared for:

- *Alexandrium* spp.
- *Pseudo-nitzschia* spp.
- *Dinophysis acuminata, Dinophysis* spp., *Prorocentrum lima*
- *Gymnodinium catenatum*
- *Karenia /Karlodinium group*
- *Azadinium spp.*
- *Rhizosolenia amaralias (*cf *chunii)*

These contingency plans will be implemented in any of the following events:

- The abundance of any potentially toxic phytoplankton species exceeds the relevant trigger levels for biotoxin testing listed in Appendix 11.
- The detection of any phytoplankton species at levels known to be toxic overseas but of unknown toxicity in Australian waters.
- The presence of biotoxins in shellfish flesh.
- Any other reason as determined by either the Victorian Fisheries Authority, Harvest Area Coordinator or PrimeSafe.

The contingency plans will be reviewed and updated as required, or immediately if any relevant new information or regulation relating to marine biotoxins in shellfish becomes available. Advice

to Harvest Area Coordinator and harvesters could be provided by PrimeSafe, Victorian Fisheries Authority, contracted laboratories and consultants, the ASQAAC or other suitable qualified experts.

# 9 Area Closure and Reopening

## 9.1 Closure Criteria

The following criteria determine whether a closure needs to be implemented:

- The abundance of potentially toxic phytoplankton species exceeds the trigger for harvest suspension pending toxin analysis (as well as that for the initiation of biotoxin analysis) as noted in Appendix 9.
- The abundance of potentially toxic phytoplankton species has not yet exceeded the warning trigger level for biotoxin testing but is approaching that level, the precautionary principle must be applied and shellfish must be sampled for biotoxins.
- Biotoxins are present in shellfish at levels equal to or over the regulatory limits noted in Appendix 10.
- Confirmed or probable cases of human illness consistent with the case definitions for PSP, NSP, DSP and ASP (Appendix 8) have resulted from the consumption of shellfish from a particular harvesting area.
- PrimeSafe, as regulator of the Food Standards Code in Victoria in respect of seafood, or the PrimeSafe Licence holder determines a closure is necessary for any other reasons (*e.g.* potential toxin producing phytoplankton species which have not previously been recorded are present).

## 9.2 Mechanism for Closure

The following procedure is useful for the closure of a harvesting area

- The Harvest Area Coordinator will close a harvesting area and PrimeSafe licence holders must cease the movement of all shellfish immediately, if any of the closure criteria mentioned above are met.
- The closure area will extend to all of the harvesting area concerned.
- Closures should be made on a precautionary shellfish species-specific (to those grown in the harvest area) basis due to differences in the abilities of various shellfish to accumulate toxins. Where several species are involved, each should be tested to determine tissue toxin levels.
- Where harvesting is suspended in a harvesting area, a closure notice will be issued within 24 hours by the Harvest Area Coordinator and communicated (e-mail or phone) to the following:
    - All PrimeSafe licence holders that participate in the shellfish harvest monitoring program for the relevant harvesting area(s).
    - PrimeSafe.
    - Victorian Fisheries Authority.

- Where the presence of biotoxins in shellfish tissue is confirmed, the public will need to be informed. Public warnings will be issued by the Public Health Division, Department of Health based on advice provided by PrimeSafe.
- A recall of commercial product will be made where necessary by PrimeSafe (Refer to Section 11).

## 9.3 Industry Instigated Closure

PrimeSafe licence holders may choose to instigate a voluntary closure based on criteria such as pending biotoxin testing results, toxins present in neighbouring harvest areas, rising levels of toxic phytoplankton, the presence of *Rhizosolenia amaralis* (bitter taste alga) or any other criterion they deem important enough to necessitate a closure.

## 9.4 Re-opening Criteria

- A shellfish harvesting area closed due to the presence of potentially toxic or unknown phytoplankton pending biotoxin analysis, may be reopened by the Harvest Area Coordinator immediately if the results of biotoxin testing prove negative.
- A shellfish harvesting area closed due to marine biotoxins shall not be reopened until the Harvest Area Coordinator has determined that each of the following requirements for reopening have been adequately addressed:
    - The edible portion of each molluscan species harvested from the closed harvesting area meets the following criteria:
        - PSP levels are less than the regulatory limit of 0.8 mg saxitoxin equivalent /kg edible shellfish flesh (80µg/100g) as determined by Liquid Chromatography Fluorescence Detection (LC-FLD) or LC-MS/MS in two successive samples from the same site taken at least 7 days apart; phytoplankton abundance not rising.
        - ASP levels are <10 mg domoic acid equivalent/kg edible shellfish flesh (20 µg/g or 20 ppm), by Liquid chromatography–mass spectrometry-mass spectrometry (LC-MS/MS), in two successive samples from the same site taken at least 7 days apart; phytoplankton abundance not rising.
        - DSP levels (not including pectenotoxin 2 seco-acids and their derivatives in mussels) are less than 0.16 mg okadaic acid equivalents/kg edible shellfish flesh (16 µg/100g) by LC-MS/MS, two successive samples from the same site taken at least 7 days apart; phytoplankton abundance not rising.
        - NSP levels are less than 200 mouse units/kg edible shellfish flesh (20MU/100g), by ether extraction and mouse bioassay with a maximum observation time of 6 hours, in two consecutive samples from the same site, taken not less than7 days apart.
    - The abundance of toxic phytoplankton relating to the toxin present has shown a clear downward trend and the cell counts are below the threshold level used to

initiate closure (Appendix 9). The Harvest Area Coordinator and PrimeSafe licence holders should consider whether the level of other potentially toxic phytoplankton species are increasing, necessitating another closure within a short time frame.

- Once below the regulatory limit, toxin levels are decreasing or static in the required number of consecutive samples (dependent on the biotoxin type) in order for the area to be re-opened.

- Other conditions or limitations may be applied if considered necessary by the designated Harvest Area Coordinator and imposed by PrimeSafe.

## 9.5 Mechanisms for Re-opening

The Harvest Area Coordinator will reopen a shellfish Harvesting Area to harvesting and movement of shellfish only when each of the reopening criteria have been met.

The Harvest Area Coordinator shall, on each reopening event, prepare documents including the data, environmental conditions and factors leading to that decision.

Resumption of harvesting may be accompanied by increased monitoring where there is a risk of a secondary bloom or low tissue biotoxin levels (less than the regulatory limit) persist.

When harvesting is recommenced in a Harvesting Area, a reopening notice will be issued by the harvest area manager and communicated (e-mail or phone) to the following:

- All PrimeSafe licence holders that participate in the shellfish harvest monitoring program within the relevant Harvesting Area(s).
- Victorian Fisheries Authority.
- PrimeSafe.

## 9.6 Surveillance of harvesting during a biotoxin closure

Surveillance of harvesting during a biotoxin closure is the responsibility of PrimeSafe under the Seafood Safety Act, 2003.

# 10 Investigation of Illness Due to Toxic Shellfish Poisoning

## 10.1 Notification

Unlike food or water borne pathogens, suspected cases of toxic shellfish poisoning (TSP) are not notifiable.

## 10.2 Investigation

Where there is evidence that TSPs are the cause of an illness, it is the responsibility of the DH to investigate potential sources of contamination/illness.

Toxic shellfish poisoning 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 may be better understood. As is the case with any epidemiological investigation the aim is the control and prevention of further TSP episodes.

All suspected cases of TSP should be investigated. The investigation should include the following foundation steps (not necessarily in the order below):

- Verification of the diagnosis of reported cases and the identification of the specific etiological agent responsible.
- Confirm that an incident exists. Check for other cases at appropriate points such as medical practices in the relevant area.
- Describe the cases in the epidemic or outbreak according to the variables of time, place and person.
- Identify the source of the agent and its mode of transmission, including the specific vehicles, vectors and routes that may have been involved.
- Identify the populations that are at an increased risk of exposure to the agent.
- Plan and implement control measures such as harvesting suspension, the issue of warnings and the implementation of recalls.
- Evaluate the control measures.

Case definitions provide a detailed description of the effects of the various TSP syndromes and are presented in Appendix 8.

## 10.3 Immediate Action for Suspected Toxic Shellfish Poisoning Cases

### 10.3.1 Closures of commercial Harvesting Areas

Where investigation indicates that toxic shellfish from PPB or WP shellfish Harvesting Areas have been the cause of illness, an immediate closure will be placed on all of the relevant Harvesting Areas.

Knowledge that the victims had consumed shellfish harvested from one or more of these areas and were suffering symptoms consistent with those from TSP, together with the presence of toxic phytoplankton species above threshold abundance trigger levels or the presence of

biotoxins in shellfish tissue, would constitute evidence indicating that the consumption of contaminated shellfish may be the cause of the incident.

Public warnings should be issued pending the results of more detailed investigations. The Public Health Division, DH, should issue these in collaboration with PrimeSafe.

### 10.3.2 Control of movement of harvested shellfish

It is the responsibility of PrimeSafe to undertake a product recall/detention where appropriate as detailed in Section 11, with the cooperation of the appropriate responsible agencies including:

- Office of the Chief Health Officer, Public Health Division, DH (Victoria).
- All PrimeSafe licence holders in the relevant harvesting area(s).

### 10.3.3 Notification

Notices shall be placed in prominent places near Harvesting Areas advising the public of the closure and to advise against consuming shellfish purchased from harvesters in the area between the dates indicated. This notification will be undertaken by the PrimeSafe in consultation with Food Safety Victoria, Public Health Division, DH (Victoria).

### 10.3.4 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:

- Office of the Chief Health Officer, Public Health Division, DH (Victoria).
- Food Safety Victoria, Public Health Division, DH (Victoria).
- PrimeSafe.
- Victorian Fisheries Authority.
- All PrimeSafe licence holders in the relevant harvesting area(s).

### 10.3.5 Sampling

A suite of shellfish tissue sampling may be necessary to facilitate the investigation of a suspected TSP incident.

- Shellfish tissue samples should be taken where available along the distribution pathway from the harvesting area to the suspected TSP sufferer. These 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.
- Biotoxin levels in shellfish from each harvesting area will be available through the biotoxin monitoring program. Additional sampling and analysis can be performed as required.
- These samples need to be of sufficient size to allow analysis for non-marine biotoxin sources of illness (such as bacterial, viral or chemical contamination) so that these sources can be eliminated as the primary cause of the suspected TSP incident.
- If microbiological testing is required, the sample shall be transported in such a way as to prevent contamination and identified/labelled appropriately.

- For cases showing gastro-intestinal symptoms, faecal samples should be requested to eliminate bacterial/viral causes of illness.

### 10.3.6 Funding

Investigation of toxic shellfish poisoning incidents and the associated sampling and testing is funded by the investigating authority/agency.

# 11 Product Control

## 11.1 Product Recall

When Harvesting Areas are closed due to the presence of marine biotoxins, and potentially contaminated shellfish have been harvested prior to closure, product will need to be recalled or detained. However, phytoplankton sampling will usually provide advance warning of any potential risk of shellfish biotoxin poisoning, allowing harvesting restrictions to be implemented before potentially contaminated shellfish are harvested. The recall will include any product harvested since the last satisfactory biotoxin and or phytoplankton sampling event and should be initiated within 24 hours of Harvest Area closure.

A food product recall is carried out to protect public health and safety. A food withdrawal may also occur as a precautionary measure prior to an official recall or for quality or similar reasons (FSANZ, 2005).

## 11.2 Objectives

*The Food Industry Recall Protocol – A Guide to Writing A Food Recall Plan and Conducting a Food Recall* (FSANZ, 2004) notes that there are three primary objectives in any food recall:

- Stop the distribution and sale of an affected product.
- Inform the statutory authorities (all recalls) and the public (consumer recalls only) of the problem.
- Effectively and efficiently remove from the marketplace any product that is potentially unsafe.

## 11.3 Responsibilities

Product detention and recall will be instigated by PrimeSafe under the Seafood Safety Act, 2003. This process details the recall processes, consumer notification, product detainment and disposal. Food Safety, Department of Health also has the power to instigate product detention and seizure, in accordance with the current *Food Industry Recall Protocol* (FSANZ, 2005).

Product recall is the responsibility of the harvesters, manufacturers, processors, distributors and retailers of affected product, in conjunction with regulators.

Clause 12 of the Food Safety Standard 3.2.2 notes that:

A food business engaged in the wholesale supply, manufacture of importation of food must:

a) *have in place a system to ensure the recall of unsafe food;*

b) *set out this system in a written document and make this document available to an authorised officer on request; and*

c) *comply with this system when recalling unsafe food.*

PrimeSafe licence holders must also prepare food recall plans in accordance with Food Industry Recall Protocol (FSANZ, 2004), again permitting efficient and effective product recall.

## 11.4 Notification

Notification of food recalls is the responsibility of the business concerned. Guidance can be provided by PrimeSafe, FSANZ or DH during the notification process.

Notification should include statutory authorities, PrimeSafe licence holders in the relevant Harvesting Area(s), the product distribution network and the public (should potentially contaminated product reach the community).

# 12 References

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# APPENDICES

# Appendix 1 – Agency and Personnel Contacts

| Agency / Contact | Responsibility | Contact Details |
|---|---|---|
| **PrimeSafe** | **Authority** | **PO Box 2057, South Melbourne, VIC 3205**<br>**150 Albert Road, South Melbourne, VIC 3205**<br>**(03) 9685 7333 (Phone)**<br>**(03)9696 5284 (Facsimile)** |
| Rick Partelle | Manager PrimeSafe Shellfish Program | 03 9685 7363<br>0417 321 663<br>03 9696 5284 (F)<br>parteller@primesafe.vic.gov.au |
| **Office of Chief Health Officer, DH** | | **Department of Health (Victoria)**<br>**GPO Box 1670N**<br>**Melbourne**<br>**VIC 3001** |
| Dr Caroline McElnay | Victoria's Chief Health Officer | 03 9096 0376<br>03 9096 9166 (F)<br>chief.healthofficer@health.vic.gov.au |
| **Department of Health (Victoria)** | | **Department of Health**<br>**Department of Health GPO Box 4541**<br>**Telephone: 1300 364 352**<br>**Melbourne 3001 (1 300**<br>**Website: www.foodsafety.vic.gov.au** |
| Rachael Poon (TBC) | Water Team 2<br>People, Operations, Legal & Regulation \| Health Regulator | 03 9456 4348<br>04037 991 063<br>rachael.poon@health.vic.gov.au (TBC) |

| Agency / Contact | Responsibility | Contact Details |
|---|---|---|
| | For notifying cases or enquire about human cases | ozfoodnetvic@health.vic.gov.au |
| **EPA Victoria** | **Marine Science - Environmental monitoring & policy** | **Centre for Environmental Sciences**<br>**Ernest Jones Drive**<br>**La Trobe University Research & Development Park**<br>**Macleod, VIC, 3085**<br>**03 8458 2300 (Phone) 03 8458 2301 (Fax)** |

# Appendix 2 – Laboratories and Contacts for Phytoplankton Enumeration & Identification

| Agency / Contact | Capability/Position | Contact Details |
|---|---|---|
| **Microalgal Services** | **Phytoplankton Identification and Enumeration** | **338 Jasper Road**<br>**Ormond VIC 3204**<br>**Office:(03) 9578 2158**<br>**Lab: 0492 871 090 (E)**<br>**web: microalgal.com.au** |
| Dr Stephen Brett (1) | Senior Botanist | (03) 9578 2158<br>algae@bigpond.net.au |
| Dr Tamsyne Smith-Harding (2) | Senior Botanist | (03) 9578 2158 |
| Sam Parker (3) | Senior Botanist | (03) 9578 2158 |
| **University of Tasmania**<br>**Institute for Marine and Antarctic Studies (IMAS)** | **Phytoplankton Identification, Electron-microscopy, Phytoplankton Culture, DNA Probes** | **Private Bag 5129, Hobart, TAS, 7001** |
| Prof Gustaaf Hallegraeff | Phytoplankton Taxonomy<br>Electron Microscopy | 03 6226 2623<br>Gustaaf.Hallegraeff@utas.edu.au |

(1) Primary Contact (2) Secondary Contact (E) Emergency Contact

# Appendix 3 – Approved Laboratories and Contacts for Marine Biotoxin Analysis of Shellfish Flesh

| Agency / Contact | Responsibility/Position | Contact Details |
|---|---|---|
| **Symbio Laboratories** | **PST (LC-FLD), DST, AST, NST (LCMSMS) (NATA Accreditation No. 2455)**<br>**Accredited for compliance with ISO/IEC 17025 - Testing.** | **2 Sirius Road, Lane Cove West NSW 2066**<br>**1300 703 166**<br>**Phone 1300 703 166**<br>**Email: admin@symbiolabs.com.au**<br>**www.symbiolabs.com.au** |
| **Analytical Services Tasmania** | **PST (LC-FLD), DST, AST, NST, AZA, YTX, Cyanotoxins (LCMSMS) (NATA Accreditation No. 5589)**<br>**Accredited for compliance with ISO/IEC 17025 - Testing.** | **18 St Johns Avenue**<br>**New Town Laboratory**<br>**Phone +61 3 6165 3300**<br>**Email: enquiries@ast.tas.gov.au**<br>**www.analyticalservices.tas.gov.au** |
| **Cawthron Institute**<br>**Biotoxin Laboratory** | **PSP, DSP, ASP, NSP, Cyanotoxins Analysis (LC-MS/MS)** | **Private Bag 2 Nelson,7040 New Zealand**<br>**98 Halifax Street East, Nelson, 7010 New Zealand**<br>**0011 643 548 2319 (Phone)**<br>**lab@cawthron.org.nz** |
| Paul McNabb | Technical Manager | 0011 643 548 2319<br>Paul.mcnabb@cawthron.org.nz |

(1) Primary Contact (2) Secondary Contact (E) Emergency Contact

# Appendix 4 – Marine Biotoxin Analytical Methods

### Symbio Laboratories

| Toxin | Method | Lower Limit of Reporting |
|---|---|---|
| PSP group<br>dcGTX2,3, C1,2, dcSTX, GTX2,3, GTX5, STX, GTX1,4, dcNeo, NEO, C3,4, Total PSP | PSP confirmation by in house method ENV132 by HPLC-FLD | 0.025 mg/Kg |
| DSP group<br>Total OA, Total Dinophysistoxin 1, 2, Pectenotoxin 2, Yessotoxin, Gymnodimine, Spirolide 1, Total Azaspiracids, AZA1, AZA2, AZA3, Total DSP | DSP confirmation by in house method ENV133 by LCMSMS | 0.025 mg/Kg |
| ASP<br>Domoic Acid | DSP confirmation by in house method ENV133 by LCMSMS | 1.0 mg/Kg |

### Analytical Services Tasmania

| Toxin | Method | Lower Limit of Reporting |
|---|---|---|
| PST Group<br>C1, C2, C3*, C4*, dcGTX1*, dcGTX2, dcGTX3, dcGTX4*, dcNEO, dcSTX, doSTX*, GTX1, GTX2, GTX3, GTX4, GTX5, GTX<br>6, NEO, STX, Total PST | PST by LC-MS/MS (Boundy Method)<br>Method 3416 | 0.01 mg/Kg |
| DSP group<br>AZA1, AZA2, AZA3, DTX1 Free, DTX1 Total, DTX2 free, DTX2 total, GYM, Homo-YTX, OA Free, OA Total, PnTx-G, PTX2, SPX1, Total DST, YTX | Lipophilic Toxins in Shellfish by LC-MS/MS<br>Method 3411 | 0.01 mg/Kg |
| ASP<br>Domoic Acid | Lipophilic Toxins in Shellfish by LC-MS/MS<br>Method 3411 | 0.05 mg/Kg |

Other services available include:

- Cawthron Institute in New Zealand can conduct all toxicity tests, using the chemical confirmatory and screening test methods.

# Appendix 5 – Phytoplankton Sampling Procedures

### Collecting phytoplankton samples using the hosepipe sampler

**Purpose:**

As noted in the VSQAP, the aim is to collect a depth integrated sample of phytoplankton for enumeration over the entire depth of the mussel lines appropriate to industry practice. This is preferred over a surface sample due to variability in the vertical distribution of phytoplankton. The sample collected will be used to enumerate any toxic phytoplankton present.

**Equipment:**

- 25 mm internal diameter hosepipe sampler of appropriate length (marked at 1m intervals and weighted at bottom end).
- Strong line attached to bottom of sampler at the weight.
- Spare bungs for hosepipe sampler.
- Clean bucket (>12L volume).
- 1L sample bottles - 1 for each sample taken plus spares.
- Labels (and Lugol's preservatives if required).
- Eskies for transporting samples.

*Care is to be taken that **ALL** equipment is attached securely to the boat.*

**Method:**

Prepare hosepipe sampler

- Make sure top end is firmly attached to the boat.
- Ensure bottom line is attached firmly both to the bottom of the hosepipe and the other end to the boat.
- Remove bung from top end (or open tap or valve if present).

Collect sample

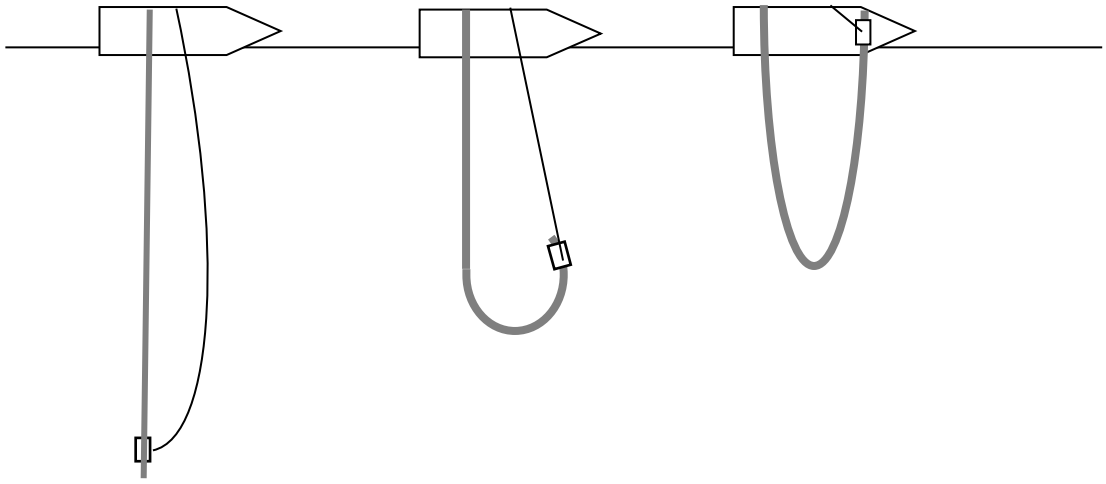
- Lower weighted, bottom end very slowly to appropriate depth, to avoid disturbing any layers of phytoplankton in the water column.
- Take care not to hit the bottom, particularly at low tide and/or where there is a swell.
- If the bottom is hit, discard the sample, clean the sampler and re-take the sample at a lesser depth.

Retrieve sample

- Replace bung securely in top of tube and pull the bottom of the hose up into the boat; make sure the bung remains firmly in place (refer diagram).
- Insert the bottom end of hosepipe into the bucket, remove the bung and empty sample into the bucket.

Fill sample bottles

- Label sample bottle(s) with time and date of sampling, sample type and harvesting area.
- Gently mix sample in bucket.
- Sub-sample by lowering a plastic, labelled 1L bottle into bucket and fill, leaving a 10 cm air space at top; cap bottle firmly.
- Fill required number of plastic bottles with sample water.
- Store samples in an esky with one icepack to keep cool (icepack is NOT in contact with samples – purpose is to keep esky and samples cool while they are transported to the laboratory).
- Generally, samples are returned to the laboratory live but some can be preserved with Lugol's iodine or other preservatives in the field. If samples are preserved, note clearly on the label.



### Collecting phytoplankton using the plankton net

**Purpose:**

To collect a concentrated phytoplankton sample along the entire depth of the mussel lines for the purposes of detecting and identifying nuisance species, including those that may be present in low numbers.

**Equipment:**

- 20μm mesh plankton net of 300mm diameter and 1 metre length.
- 75 mL polycarbonate bottle at base of plankton net.
- 125 mL plastic sample storage vials.
- Labels.
- Lugol's iodine or other preservative if required.

- Weight to attach to plankton net to facilitate sinking.

**Method:**

Check equipment

- Ensure net line is firmly attached both to the net and the boat.
- Wash net and bottle prior to use.
- Screw or otherwise attach plastic bottle onto net.
- Label and prepare 125 mL sample storage vials.

Collect sample

- Lower net to an appropriate depth.
- Do not allow the net weight to hit the bottom (clean net and repeat sample if it does).
- **Slowly** but steadily pull the net up to the boat.
- Wash material adhering to inside of net towards the net bottle end by gently dipping and shaking the net.

Fill and store sample bottles

- Carefully remove 75mL bottle from the net end.
- Transfer sample to the labelled 125mL storage vial leaving a 10 - 20mm air space.
- Cap tightly and store with other algal samples in an esky with a single ice pack (ice pack is NOT in contact with samples – purpose is to keep esky cool while samples are transported to the laboratory).
- If further samples are required, wash net and repeat as above.
- Generally, samples are returned to the laboratory live but some can be preserved with Lugol's iodine or other preservatives in the field. If samples are preserved, note on the label clearly.
- Wash the plankton net and net bottle prior to leaving the site or taking additional samples.
- Wash plankton net and sample bottle with freshwater and dry fully prior to storage.

# Appendix 6 – Phytoplankton Species Lists

The following lists are presented to summarise the phytoplankton species that potentially produce biotoxins and present a potential risk of human illness resulting from the consumption of shellfish contaminated with these toxins. It is stressed that the tables are "all inclusive" and that there is great variability in the level of evidence resulting in the inclusion of species as potentially toxic. This evidence varies from that which is circumstantial at best (*e.g.* species was present during a single incident at one locality which had several potential causes, one of which was biotoxins) to very powerful evidence of widespread toxicity supported by detailed biotoxin studies. The tables are presented as a guide, and it is crucial that they be modified to incorporate local and international information as it comes to hand and that management decisions are made with full awareness of why a species was listed or unlisted as potentially toxic.

Nonetheless, all records of toxicity should be examined carefully as the toxicity of specific algal species may vary substantially between different geographical areas and even from time to time in the same area. In addition, there are records of the introduction of new forms in recent years through agents such as ballast water *e.g. Gymnodinium catenatum* into Southeast Tasmanian waters. The potential therefore exists for the introduction of toxic species or strains not seen in an area previously.

Categories A2 – C are essentially reproduced from the *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001).* In addition, detailed records of phytoplankton occurrence and biotoxin presence in shellfish have been collected as part of the Victorian Shellfish Quality Assurance Program extending back to 1987. This together with other information presented in the literature has permitted the presentation of an additional list of potentially toxic phytoplankton specific to the regions of Port Phillip Bay and Western Port containing shellfish harvest areas. This is presented as Category A1 and is modified from Category A as presented in the *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001)*. It is stressed that this list should only be used in relation to those harvest areas covered by the Victorian Marine Biotoxin Management Plan to date, Port Phillip Bay and Western Port. Phytoplankton listed as Category A1 may also be included in the Category A2 list.

**Category A1:** Species occurring in south-eastern Australian waters, which are known or suspected toxin producers in Australia.

| Species | Toxins/Comments |
|---|---|
| **Bacillariophyceae (Diatoms)** | |
| *Pseudo-nitzschia australis* | ASP (domoic acid) |
| *Pseudo-nitzschia multiseries* | ASP (domoic acid) |
| *Pseudo-nitzschia delicatissima* | NT in PPB & Tas<br>ASP (domoic acid) overseas |
| *Pseudo-nitzschia galaxiae* | NT in all PPB isolates so far |
| *Pseudo-nitzschia turgidula* | ASP (domoic acid); NT Australia, weakly toxic NZ |
| *Pseudo-nitzschia fraudulenta* | ASP (domoic acid); NT Australia, weakly toxic NZ |
| *Pseudo-nitzschia pungens* | NT in PPB and Bass Strait<br>Toxic strains elsewhere incl. NZ - ASP (domoic acid) |
| *Pseudo-nitzschia pseudodelicatissima* | NT in PPB, Vic, NSW<br>One of main bloom species in PPB, Vic and Tas<br>Toxic strains elsewhere? - ASP (domoic acid) |
| *Pseudo-nitzschia multistriata* | NT in Aust? Very common.<br>ASP (domoic acid) New Zealand (weakly toxic) |
| **Dinophyceae (dinoflagellates)** | |
| *Alexandrium catenella* | PSP (Saxitoxins, $C_1$ - $C_4$, gonyautoxins) |
| *Alexandrium pacificum* | PSP (Previously identified as *A.catanella* in Vic waters) |
| *Alexandrium australiense* | NT in all Australian isolates so far – some toxic strains?<br>PSP (Saxitoxins, $C_1$ - $C_4$, gonyautoxins) |
| *Alexandrium fundyense* | *A. fundyense* from PPB shown to be *A. catenella* |
| *Alexandrium minutum* | PSP (Saxitoxins, mostly gonyautoxins) |
| *Alexandrium ostenfeldii* | Not linked to toxicity in Aust; non-bloom forming<br>Sometimes toxic NZ – saxitoxins and derivatives<br>Canada - spirolides |
| *Alexandrium insuetum* | NT in all PPB isolates so far |
| *Dinophysis acuminata* | DSP - Weakly toxic in NZ<br>OA , ?DTX 3 (not tested yet) |
| *Dinophysis caudata* | ?DSP (?OA, ?DTX 1 – 3) |
| *Dinophysis fortii* | ?DSP (?OA, ?DTX 1 – 3) |
| *Dinophysis acuta* | ?DSP (?OA, ?DTX 1 – 3); DSP in NZ |
| *Dinophysis miles* | ?DSP (?OA, ?DTX 1 – 3) |
| *Dinophysis tripos* | ?DSP (?OA, ?DTX 1 – 3) |
| *Prorocentrum lima* | ?DSP (?OA, ?DTX 1 – 3) |
| *Gymnodinium catenatum* | PSP (sulphamate saxitoxins) |
| *Karenia cf brevis* | ?NSP (BTX) |
| *Karenia mikimotoi* | ?NSP – NR of toxicity in Aust to date<br>Non BTX producer in NZ; Gymnocin in Japan |

NT = Non Toxic  PPB =  Port Phillip Bay  Vic = Victoria  Tas = Tasmania  NZ = New Zealand  Aust = Australia
OA = Okadaic acid  DTX = Dinophysis toxins  DTX3 = diol esters  BTX = brevetoxins
? Indicates this toxin has not been confirmed in Australian strains of this species, at the time of this report.

**Category A2:** Species known to be present Australian waters and proven to produce toxins either in Australia or internationally. (Modified from *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001)*

| Species | Toxins/Comments |
|---|---|
| *Pseudo-nitzschia australis* | ASP (domoic acid) |
| *Pseudo-nitzschia delicatissima* | ASP (domoic acid) |
| *Pseudo-nitzschia fraudulenta* | ASP (domoic acid); NT Australia, weakly toxic NZ |
| *Pseudo-nitzschia multiseries* | ASP (domoic acid) |
| *Pseudo-nitzschia pseudodelicatissima* | ASP (domoic acid) |
| *Pseudo-nitzschia pungens* | ASP (domoic acid)<br>Usually NT but some strains produce high ASP levels<br>ASP (domoic acid) |
| *Pseudo-nitzschia turgidula* | ASP (domoic acid); NT Australia, weakly toxic NZ |
| *Alexandrium pacificum* | PSP (saxitoxin and derivatives) |
| *Alexandrium catanella* | PSP (saxitoxin and derivatives) |
| *Alexandrium minutum* | PSP (saxitoxin and derivatives) |
| *Alexandrium ostenfeldii* | PSP (saxitoxin and derivatives)<br>Spirolides in Canada |
| *Alexandrium tamarense* | PSP (saxitoxin and derivatives)<br>Also has non-toxic strains |
| *Dinophysis acuminata* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis acuta* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis caudata* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis fortii* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis hastata* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis mitra* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis rotundata* | DSP (OA?, DTX 1 – 3?) |
| *Dinophysis tripos* | (DSP (OA?, DTX 1 – 3?)<br>Some strains only |
| *Gymnodinium catenatum* | PSP (saxitoxin and derivatives) |
| *Karenia cristata* | NSP (brevetoxins) |
| *Karenia* cf *brevis* | NSP (brevetoxins) |
| *Gymnodinium aureolum* | ?NSP (?weakly toxic in NZ) |
| *Karlodinium micrum* | ?NSP (?weakly toxic in NZ); fish killer |
| *Prorocentrum lima* | DSP (OA?, DTX 1 – 3?) |
| *Pyrodinium bahamense* var. *compressum* | Tropical habitats<br>PSP (saxitoxin and derivatives) |

NT = Non Toxic ? DTX 3 = OA esters Indicates this toxin has not been confirmed in Australian strains of this species, at the time of this report .

**Category B**: Potential toxin producing species *(i.e.* toxicity untested/unclear) known to be present in Australian coastal waters including species known/suspected to be toxic overseas (Modified from *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001).*

| Species | Toxins/Comments |
|---|---|
| *Azadinium spp.* | Possibly AZA1-3 Toxic in New Zealand |
| *Alexandrium fraterculus* | Possibly PSP (saxitoxin and derivatives) |
| *Alexandrium fundyense* | Possibly PSP (saxitoxin and derivatives) |
| *Coolia monotis* | Cooliatoxin |
| *Pseudo-nitzschia cuspidata* | Possibly ASP (domoic acid) |
| *Pseudo-nitzschia heimii* | Possibly ASP (domoic acid)<br>Non-toxic in New Zealand; toxicity unknown elsewhere |
| *Pseudo-nitzschia lineola* | Possibly ASP (domoic acid) |
| *Pseudo-nitzschia multistriata* | Possibly ASP (domoic acid)<br>Non-toxic in New Zealand |
| *Pseudo-nitzschia subfraudulenta* | Possibly ASP (domoic acid) |
| *Pseudo-nitzschia subpacifica* | Possibly ASP (domoic acid) |
| *Alexandrium pseudogonyaulax* | Possibly PSP (STX and derivatives, goniodomin) |
| *Chattonella marina/antiqu* | Possibly NSP (brevetoxins) |
| *Fibrocapsa japonica* | Possibly NSP (brevetoxins) |
| *Heterosigma akashiwo* | Possibly NSP (brevetoxins) |
| *Karenia papilionacea* | Possibly NSP (brevetoxins) |
| *Karenia selliformis* | Gymnodimine and low BTX levels - New Zealand |
| *Prorocentrum concavum* | DSP (OA?, DTX 1 – 3?) |

NT = Non Toxic  STX = saxitoxin

**Category C:** Other potential toxin producing species world-wide that may be present in Australian waters (Modified from *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001).*

| Species | Toxins/Comments |
|---|---|
| *Alexandrium angustitabulatum* | Possibly PSP (saxitoxin and derivatives)<br>Present in New Zealand |
| *Alexandrium acatenella* | Possibly PSP (saxitoxin and derivatives) |
| *Alexandrium cohorticula* | Possibly PSP (saxitoxin and derivatives) |
| *Alexandrium lusitanicum* | Possibly PSP (saxitoxin and derivatives) |
| *Alexandrium tamiyavanichi* | Possibly PSP (saxitoxin and derivatives) |
| *Dinophysis norvegica* | Major DSP producer in Europe |
| *Gymnodinium aureolum* | Possibly NSP (brevetoxins)<br>Low levels of BTX in New Zealand; NT in Aust? |
| *Gymnodinium impudicum* | Possibly NSP (brevetoxins)<br>Low levels of BTX in New Zealand? |
| *Gymnodinium pulchellum* | Possibly NSP (brevetoxins)<br>NT in PPB and Aust? |
| *Karenia bidigitata* | Possibly NSP (brevetoxins)<br>Low levels of BTX in New Zealand? |
| *Karenia brevisulcata* | Wellington Harbour Toxin (WHT)<br>Low levels of BTX in New Zealand? |
| *Karlodinium micrum* | Possibly NSP (brevetoxins) – low BTX levels in NZ |
| *Lingulodinium polyedra* | Yessotoxins in Japan |
| *Nitzschia navis-varingica* | ASP(domoic acid) in brackish Vietnamese waters |
| *Ostreopsis siamensis* | Ostreotocin |
| *Pfiesteria piscicida* | Toxin being characterised |
| *Prorocentrum elegans* | DSP (OA?, DTX 1 – 3?) |
| *Prorocentrum hoffmannianum* | DSP (OA?, DTX 1 – 3?) |
| *Prorocentrum maculosum* | 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 |

NT = Non Toxic PPB = Port Phillip Bay BTX = brevetoxin DTX 3 = OA esters
? Indicates this toxin has not been confirmed in Australian strains of this species, at the time of this report .

**Category D:** Nuisance species known to be present in Australian waters that are not known to be toxic to humans but are to be monitored for other reasons including potential for economic damage to industry and its reputation (Modified from *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001).*

| Species | Toxins/Comments |
|---|---|
| *Rhizosolenia amaralis (*formerly cf *chunii*) | NT but produces a bitter taste in mussels, oysters and scallops in PPB. |

# Appendix 7 – 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
- Any of the following neurological symptoms occurring within 24 hours of consuming shellfish:
    - Neurosensory symptoms
        - 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 symptoms
        - Weakness such as trouble rising from seat or bed
        - Difficulty swallowing
        - Difficulty breathing
        - Paralysis
        - Clumsiness
        - Unsteady walking
        - Dizziness/vertigo
        - Slurred/unclear speech
        - Double vision
- 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/neuro-cerebellar 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 within 7 days of the collection of shellfish consumed by the case, PSP biotoxins are detected at or above the regulatory limit (currently 80 µg/100 g tissue) in shellfish obtained from near or at the same site (not leftovers).

### Confirmed case

- Meets the clinical case definition
- AND PSP biotoxins are detected 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).

## *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 the consumption of the shellfish:
    - Confusion
    - Memory loss
    - Disorientation
    - Seizure
    - Coma

### Probable case

- Meets the clinical case definition
- And within 7 days of the collection of shellfish consumed by the case ASP biotoxins are detected at or above the regulatory limit (currently 20 ppm domoic acid/100 g tissue) in shellfish obtained from near or at the same site (not leftovers).

### Confirmed case

- Meets the clinical case definition
- And ASP biotoxins detected 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 within 7 days of collection of shellfish consumed by the case, DSP biotoxins are detected at or above the regulatory limit (currently 20 µg/100 g shellfish or 5 MU/100 g) in shellfish obtained from near or at the same site (not leftovers).

#### 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).

## *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 within 7 days of collection of shellfish consumed by the case, NSP biotoxins detected at or above the regulatory limit (currently 20 MU/100 g shellfish) in shellfish obtained from near or at the same site (not leftovers).

### Confirmed case

- Meets the clinical case definition
- Detection of NSP toxins 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).

# Appendix 8 – Phytoplankton Action Levels

The following table summarises the phytoplankton levels (in cells/litre) that are used to trigger the sampling of shellfish flesh for biotoxin analysis and harvesting suspensions. These levels are derived from levels used internationally and in various States in Australia. They have been modified in accordance with specific information obtained pertaining to phytoplankton presence/abundance and biotoxin levels in shellfish tissue as part of shellfish quality assurance monitoring, in Port Phillip Bay and Western Port. They should be further revised as additional monitoring and research is undertaken and supports a change.

Note: For *Pseudo-nitzschia spp* risk remains high for a minimum of two weeks post bloom crash.

| Phytoplankton Abundance Triggers for this Victorian Marine Biotoxin Management Plan (cells/L) | | | | | |
|---|---|---|---|---|---|
| **Alga / Algal Group** | **Toxin** | **Definitive Identification & Warning to Growers** | **Tissue Testing** | **Harvest Suspension Pending Toxin Analysis** | **Harvest Resumption Refer section 9.4 Re-opening criteria** |
| **Bacillariophyceae** | | | | | |
| *Pseudo-nitzschia* spp. (*pseudodelicatissima* group)** | ASP (domoic acid) | 100,000 | 300,000 | 500,000 | <20 mg/kg domoic acid for 2 successive samples taken at least 7 days apart; phytoplankton abundance not rising. |
| *Pseudo-nitzschia* spp. *australis, pungens* & *multiseries* (in *seriata* group) | ASP | 100,000 | 100,000 | 300,000 | As Above |
| *Rhizosolenia amaralis* (*imbricata* group) | Bitter Taste | 10,000 | N/A | 20,000 Level 2 Warning | Harvesting suspended/resumed by growers depending on taste of mussels. |
| **Dinophyceae**<br>*Alexandrium catenella*<br>*Alexandrium pacificum*<br>*Alexandrium minutum*<br>*Alexandrium australiense* | PSP<br><br>PSP (some strains) | 200 | 200 | 500 | <0.8 mg/kg PSP for 2 successive samples at least 7 days apart; phytoplankton abundance not rising. |
| *Alexandrium* spp. *(if unknown or in doubt) (A.pseudogonyaulax, A.margalefii and A.insuetum* not known to be toxic in Aus waters) | PSP Some strains | 200 | 200 | 500 | As Above |
| *Azadinium* spp. | AZA1-3 | 30,000 | 30,000 | 30,000 | Precautionary limit same as NZ limit |
| *Gymnodinium catenatum* | PSP | 1,000 | 1,000 | 5,000 | <0.8 mg/kg PSP for 2 successive samples taken at least 7 days apart; phytoplankton abundance not rising. |
| **Dinophysis acuminata* | DSP | 1,000 | 1,000 | 2,000 | <0.20 mg/kg DSP for 2 successive samples taken at least 7 days apart; phytoplankton abundance not rising. |
| *Dinophysis caudata*<br>*Dinophysis fortii*<br>*Dinophysis acuta* | DSP | 1,000<br><br>500 | 1,000<br><br>500 | 2,000<br><br>1,000 | As Above |
| *Dinophysis* spp. | ?DSP | 500 | 500 | 1,000 | As Above – precautionary only till further information available |
| *Karenia brevis* (Not currently recorded in Aust)<br>*Karenia cristata**** | NSP brevetox in (BTX) | 1,000 | 2,000 | 5,000 | < 0.8 BTX-2 eq for 2 successive samples taken at least 7 days apart; phytoplankton abundance not rising. |
| *Karenia mikimotoi, K. papilionacea, K. bidigitata, K. brevisulcata, K. selliformis*<br>Flat, Australian species morphologically similar to *K. brevis* or *K. mikimotoi*<br>*Karlodinium micrum, Gymnodinium impudicum* | ?NSP | 100,000 | 250,000 | 300,000 | < 0.8 BTX-2 eq for 2 successive samples taken at least 7 days apart; phytoplankton abundance not rising. |

| | | | | | |
|---|---|---|---|---|---|
| *Prorocentrum lima* | ?DSP | 500 | 500 | 1000 | |

* Draft *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming* (2001) trigger adopted for now until more information on DTX-3 (OA esters) is available for PPB; PTX2-SA no longer included as toxins.
NOTE: Harvest suspension pending biotoxin analysis is precautionary; suspension / resumption of harvesting will be determined by toxin levels and their regulatory limit as noted below.
**Unless these *Pseudonitzschia* species are distinguished definitively from the lower toxicity group (which cannot be done with analysis by light microscopy) the lower trigger levels as specified for the *P. australis* group must be applied.
*** *Karenia cristata* was first identified in South Australia 2025 harmful algal bloom. Initial indications are it contains similarly high levels of brevetoxin as *K.brevis*. Trigger levels may be altered as more information arises.

# Appendix 9 – Marine Biotoxin Regulatory and Advisory Levels

The following table shows the maximum levels for each regulated marine biotoxin group as required by the FSANZ Food Standards Code (2005), and the maximum levels applied in the Victorian Marine Biotoxin Management Plan for toxins that are not regulated in the Food Standards Code (yellow shading).

**Victorian Marine Biotoxin Management Plan maximum levels by biotoxin group.**

| Toxin Class | Units | Regulatory Limit[a] | Method | Limit of Detection | Laboratories Utilised |
|---|---|---|---|---|---|
| PSP | mg/kg | 0.8 STX eq | LC-FLD<br>LC-MS/MS | 0.05 | Symbio Laboratories / Analytical Services Tasmania |
| ASP (domoic acid) | mg/kg | 20 | LC-MS/MS<br>LC-MS/MS | 0.025 | Symbio Laboratories / Analytical Services Tasmania |
| DSP | mg/kg | 0.16 [b] | LC-MS/MS | 0.025 | Symbio Laboratories / Analytical Services Tasmania |
| NSP | mg/kg<br>MU/kg[c] | 0.8 BTX-2 eq<br>200 | LC-MS/MS<br>Mouse Bioassay | 0.1 – 0.2<br>10 | Symbio Laboratories /Cawthron Institute (NZ) / Analytical Services Tasmania |
| Yessotoxins (YTX) | mg/kg | 1.0 | LC-MS/MS | 0.025 | Symbio Laboratories / Analytical Services Tasmania |
| Azaspiracids (AZA) | mg/kg | 0.16 | LC-MS/MS | 0.025 | Symbio Laboratories / Analytical Services Tasmania |

[a] PSP, ASP, DSP & NSP regulatory limits from FSANZ Food Standards Code (2005). Yessotoxins and azaspiracids are not currently regulated in Australia under the Food Standards Code.
[b] DSP toxins include okadaic acid, dinophysistoxins (DTX1, DTX2, DTX3) and pectenotoxins. Pectenotoxin-2 seco acid is not included.
[c] MU = mouse units

## Notes on detection and quantification of biotoxin levels:

### Toxins regulated under the Food Standards Code:

#### Paralytic Shellfish Poisoning (PSP)

Analysis is by Liquid chromatography– Fluorescence Detector (LC-FLD) or Liquid chromatography–mass spectrometry/mass spectrometry LC-MS/MS. The maximum level is PSP toxins greater than or equal to 0.8 mg of saxitoxin equivalents/kg of edible shellfish flesh determined by the sum of the toxicity equivalent factors (TEFs) for all individual PSP toxins. The Food Standards Code does not specify the toxicity equivalence factors. The Victorian Marine Biotoxin Management Plan utilises the TEFs specified in Oshima (1995)[1] with the exception of the TEF for neo-saxitoxin, for which a TEF of 2.54 is utilised. This is a precautionary measure based on the studies of oral toxicity of Paralytic Shellfish Toxins

[1] Oshima, Y. (1995) *Postcolumn derivatization liquid chromatographic method for paralytic shellfish toxins.* Journal of AOAC International 78(2): 528 - 532.

undertaken by Munday et al. (2013)[2], which showed that the oral toxicity of neo-STX was significantly higher than that previously assessed by intraperitoneal injection.

### Amnesic Shellfish Poisoning (ASP)

Analysis is by Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). The maximum level is greater than or equal to 20 mg/kg of domoic acid and its isomers in the edible shellfish flesh. No toxicity equivalent factors are set for the isomers of domoic acid, which for regulatory purposes are assumed to be equipotent to domoic acid.

### Neurotoxic Shellfish Poisoning (NSP)

Historically a maximum level of NSP toxins greater than or equal to 200 mouse units/kg of edible shellfish flesh was applied, using analysis by ether extraction and mouse bioassay with a maximum observation time of 6 hours. Currently analysis of brevetoxin (BTX-1, BTX-2 & BTX-3) levels is undertaken by Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). The maximum level applied to the results of this analysis is measured in BTX-2 equivalents (i.e. 0.8mg/kg BTX-2 equivalents/kg shellfish), which is considered to be equivalent to 200 mouse units/kg.[3] No toxicity equivalent factors have been set for brevetoxins, which for regulatory purposes are deemed to be equipotent to BTX-2.[4]

### Diarrhetic Shellfish Poisoning (DSP)

DSP toxins include OA, DTX1, DTX2, DTX3 and PTX, but do not include Pectenotoxin-2 seco acids, Yessotoxins, Gymnodimine or Azaspiracids. Analysis is undertaken by Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). A maximum level of greater than or equal to 0.16 mg OA eq/kg of edible shellfish flesh is applied. FSANZ concluded that the Code should be amended to align the MLs for diarrhetic shellfish toxin in bivalve molluscs with the MLs established by Codex.

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## Toxins not regulated in Australia under the Food Standards Code

### Yessotoxins (YTX)

Yessotoxins include YTX, 45OH-YTX, Homo-YTX and 45OH Homo-YTX. Analysis is undertaken by Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). A maximum level of greater than or equal to 3.75 mg YTX eq/kg of edible shellfish flesh is applied.

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[2] Munday, R., Thomas, K., Gibbs, R., Murphy, C. & Quilliam, M.A. (2013) *Acute toxicities of saxitoxin, neosaxitoxin, decarbamoyl saxitoxin and gonyautoxins 1&4 and 2&3 to mice by various routes of administration*. Toxicon 76:77-83.

[3] http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm374275.htm). http://www.issc.org/client_resources/2011%20summary%20of%20actions/with%20fda%20concurrence/proposal%2009-101.pdf

[4] Based on historic data the risk of brevetoxins in Victoria is extremely low. However should they be detected the TEFs may be revised based on the toxicological information available at the time.

### Azaspiracid Shellfish Poisoning (AZP)

The Azaspiracid group includes AZA-1, AZA-2 and AZA-3. Analysis is undertaken by Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). A maximum level of greater than or equal to 0.16 mg AZP/kg of edible shellfish flesh is applied. This is consistent with the recommendations in Codex Standard 292-2008 (revised 2014).

# Appendix 10 – Nuisance/Toxic Phytoplankton Management Protocols

***Alexandrium* spp.**

***Gymnodinium catenatum***

***Dinophysis acuminata, Dinophysis* spp., *Prorocentrum lima***

***Pseudo-nitzschia* spp.**

***Rhizosolenia chunii***

***Karenia / Karlodinium group***

## *Alexandrium* spp.

**BACKGROUND**

*Alexandrium* spp. are small, armoured dinoflagellates. The latter are golden-brown algae with a large nucleus. They have two flagella, one protruding from a horizontal girdle groove and the other from a vertical sulcus groove (Hallegraeff, 2002).

A number of species of *Alexandrium* have been found to produce a range of toxins grouped as Paralytic Shellfish Poisons (PSPs) that may be accumulated in the flesh of shellfish. PSPs may be fatal to human consumers of contaminated shellfish through respiratory paralysis although this is rare, and there have been no fatal cases in Australia. It should be noted that toxicity within a species may be variable both with locality and time. It is stressed that some *Alexandrium* species are difficult to identify definitively, and expert assistance should be sought where doubt exists. Until definitive identification is obtained, it should be assumed that all the forms of *Alexandrium* present are toxic.

The symptoms of paralytic shellfish poisoning include numbness, dizziness, nausea, tingling in the extremities, vomiting and diarrhoea in mild cases (within 30 minutes), to choking sensations, breathing difficulties and death from respiratory paralysis 2 – 24 hours after ingestion in severe cases (Hallegraeff, 1997).

Very high levels of *Alexandrium pacificum* have resulted in highly toxic shellfish (including wild mussels) in Port Phillip Bay in the past. This species, now identified as *A.pacificum* was previously recorded as *Alexandrium catanella* in Victorian waters. *A.catanella* is another toxic species found elsewhere around the world. This coupled with the nature of the toxin, results in this group of algae presenting a substantially greater potential threat to human health than all other potentially toxic species in these waters. However, it should be noted that most of the previous blooms of *Alexandrium* did not occur in the vicinity of any of the shellfish growing areas. The most susceptible area has been Hobson's Bay near the mouth of the Yarra River, and the main public health threat was from the recreational harvesting of mussels. Past monitoring included both the Victorian Shellfish Quality Assurance Program (VSQAP) and additional bay wide monitoring funded by the Health Department. The latter no longer occurs and due to the separation between the mussel harvesting areas and the more susceptible recreational areas nearer to the Yarra River, it is unlikely that this Victorian Marine Biotoxin Management Plan and VSOM monitoring will provide any warning of the presence of *Alexandrium* in the latter. PSP was detected in mussels from the Clifton Springs and Grassy Point harvesting areas in 1993 and 1994. *A. australiense (tamarense)* was considered the most likely source of PSP in the winter of 1993 (Arnott et al, 1999).

The *Alexandrium* spp. known from Port Phillip Bay and Western Port in Victoria include the following. Those of major concern are the three PSP producing species. Several of these are new records for these areas being detected for the first time by phytoplankton monitoring.

Additional information concerning toxigenic species of *Alexandrium* in Australia may be found in Hallegraeff *et al* (1991) and Hallegraeff (2002). Similar information for New Zealand may be found in Chang (2004) and Rhodes (2005).

| | |
|---|---|
| *Alexandrium catenella* | PSP ($C_1$ – $C_4$, gonyautoxins); |
| *Alexandrium pacificum* | PSP, previously identified as *A.catanella* in Victorian waters. Present in Port Phillip Bay |
| *Alexandrium australiense* (previously *tamarense)* | Some strains PSP ($C_1$ – $C_4$, gonyautoxins); can be toxic but NT in all Australian isolates so far; present in Port Phillip Bay. |
| *Alexandrium fundyense* | PSP ($C_1$ – $C_4$, gonyautoxins); Port Phillip Bay material has been shown to be *A. catenella* |
| *Alexandrium minutum* | PSP (mainly gonyautoxins); high PSP levels in SA; has bloomed in Port Phillip Bay in winter. |
| *Alexandrium ostenfeldii* | Sometimes toxic in NZ, probably non-toxic in Port Phillip Bay & Aust; non blooming species |
| *Alexandrium pseudogonyaulax* | Non-toxic |
| *Alexandrium concavum* | Non-toxic, Port Phillip Bay, rare |
| *Alexandrium insuetum* | Non-toxic, Port Phillip Bay |
| *Alexandrium peruvianum* | Non-toxic, Port Phillip Bay |
| *Alexandrium affine* | Non-toxic, may occur in Port Phillip Bay |
| *Alexandrium margalefi* | Non-toxic, may occur in Port Phillip Bay |

**MANAGEMENT PROTOCOL**

The following management protocol has been designed to facilitate the safe harvest of mussels from monitored Harvesting Areas in Port Phillip Bay (PPB) and Western Port (WP) for human consumption, and that harvesting does not occur when the mussels are affected by toxins. The protocol is based on the following key factors:

- A number of species of *Alexandrium* occur naturally in Port Phillip Bay and Western Port.
- Definitive identification of the various species of *Alexandrium* may be difficult.
- *A. catenella* has bloomed several times in the past in Hobson's Bay (northern Port Phillip Bay) although not in the vicinity of the shellfish harvesting areas.
- *A. tamarense* may have been responsible for the presence of PSP in mussel tissue in PPB in the past.
- Extreme PSP intoxication is potentially lethal to human beings.
- The potentially toxic species *A. pacificum, A. minutum* and *A. australiense* have been detected in PPB.
- Due to the status of PPB as a harbour and the presence of a substantial number of foreign species probably introduced through ballast water, there is a danger that other toxic forms will be introduced.
- Routine phytoplankton sampling for PSP producing phytoplankton will continue to occur at all Victorian Harvesting Areas.
- Analysis of mussel tissue for PSP will be undertaken if phytoplankton numbers exceed the specified phytoplankton trigger levels for biotoxin testing.

The following management protocol has been modified from the methodology used successfully by the VSQAP between August 1999 and December 2008, where routine PSP biotoxin analyses were performed at each Harvesting Area in Port Phillip Bay and Western Port. Within the VSQAP, PSP biotoxin testing will be performed when phytoplankton abundance triggers indicate this is necessary, as for other biotoxins. The VMBMP has been modified to incorporate this change in monitoring methods for the Port Phillip Bay and Western Port shellfish Harvesting Areas. It should be noted that the principal trigger for harvest suspension is the biotoxin level in shellfish tissue; phytoplankton abundance forms an additional, early warning trigger allowing precautionary closure pending biotoxin results.

1. Phytoplankton samples are taken routinely at all Victorian Harvesting Areas.
2. If potential toxin producing species (or unknown species) of *Alexandrium* are detected in a routine sample at an abundance of >200 cells/L (2 cells/mL), the following actions must be undertaken:
    a. A warning must be issued to all relevant harvesters.
    b. The sampling frequency must be reviewed with a view to increasing it to provide the relevant data.
    c. A tissue sample be collected and sent for PSP analysis immediately.
3. If sampling frequency is not increased, then harvesting should be suspended pending the results of the next routine sampling event; previous history shows that the numbers of the phytoplankton can increase very rapidly. A live phytoplankton sample (preferably concentrated) is to be sent by overnight courier to a suitably qualified expert on this group *e.g.* Prof Gustaff Hallegraeff at the University of Tasmania for definitive identification. Advise the recipient in advance that the sample has been despatched.
4. Where doubt exists as to the identity of the form of *Alexandrium* present, toxicity should be assumed until biotoxin levels are known.
5. Where *Alexandrium* species are detected in numbers >500cells/L, harvesting should be suspended pending the results of tissue testing. The relevant analytical laboratory should be advised of the phytoplankton result and the urgency of the situation.
6. Where tissue is found to contain PSPs at a level exceeding 0.8 mg/kg (80 µg/100g) tissue (the regulatory limit), harvesting is to be suspended and is to remain suspended until two successive samples taken at least 7 days apart reveal toxin levels < 0.8 mg/kg tissue.
7. Where lower levels of toxin are detected during the growth phase of a bloom, harvesting should be suspended, and sampling frequency increased to monitor the development of the bloom.
8. Where toxin levels have exceeded the 0.8 mg/kg tissue regulatory limit during a bloom, but the bloom is clearly degenerating, harvesting may be resumed once toxin levels remain less than 0.8 mg/kg for two successive samples taken at least 7 days apart.
9. If any toxin producing *Alexandrium* species are present, and/or low PSP levels are detected, the frequency of sampling should be reviewed and amended to ensure that it is adequate to detect changes in either phytoplankton or biotoxin levels in an effective and timely manner.

10. When harvesting is suspended during a toxic bloom, the sampling frequency may be reduced to that required by the monitoring program to save resources and costs. However, two "clear" biotoxin results ("clear" = PSP levels < 0.8 mg/kg tissue) taken at least 7 days apart are required before harvesting can resume.
11. Once *Alexandrium* and PSP toxins are undetectable, the normal sampling regime may be resumed.

**VMBMP Phytoplankton Abundance Threshold Levels (cells/L)**

| Phytoplankton Species | Toxin | Warning Issued to Growers | Tissue Testing | Harvest Suspension (Pending Toxin Analysis) | Harvest Resumption Refer section 9.4 Re-opening criteria |
|---|---|---|---|---|---|
| *Alexandrium catenella* | PSP | 200 | 200 | 500 | <0.8 mg/kg PSP for 2 successive samples at least 7days apart and phytoplankton abundance not rising. |
| *Alexandrium minutum* | PSP | 200 | 200 | 500 | As Above |
| *Alexandrium pacificum* | PSP | 200 | 200 | 500 | As Above |
| *Alexandrium australiense* (prev. *tamarense)* | PSP Some strains | 200 | 200 | 500 | As Above |
| *Alexandrium* spp. (unknown or in doubt) | PSP Some strains | 200 | 200 | 500 | As Above |
| **PSP regulatory limit:** | 0.8 mg saxitoxin equivalents/kg tissue | | | | |

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## *Gymnodinium catenatum*

**BACKGROUND**

*Gymnodinium spp.* are small, unarmoured dinoflagellate phytoplankton (golden-brown algae with a large nucleus). They have two flagella, one protruding from a horizontal girdle groove and the other from a vertical sulcus groove (Hallegraeff, 2002).

Many species belonging to the genus *Gymnodinium* are common in Australian and New Zealand waters and over 21 species have been recorded from Port Phillip Bay alone. When in bloom, a number of species within this group are fish killers, particularly where fish cannot avoid the blooms. Despite this, the vast majority of these species don't appear to cause any adverse reactions in humans who consume shellfish from bloom areas in Australia or New Zealand. In south-eastern Australia, only one species of *Gymnodinium* has been found to be toxic to humans.

It is stressed that the taxonomy of this group is poorly understood although this situation has improved recently with an increase in the research being carried out in New Zealand and Australia. *G. catenatum* is a chain forming dinoflagellate which can be recognised under the light microscope. Care should be taken not to confuse it with *Alexandrium catenella*, another chain forming dinoflagellate. Under suboptimal conditions, *G. catenatum* may be present as single cells in low numbers. These may require expert assistance to identify. Identification is best done with live material.

*Gymnodinium catenatum* produces sulphamate saxitoxins that accumulate in shellfish such as mussels and oysters. This may cause Paralytic Shellfish Poisoning (PSP) in humans who consume contaminated shellfish. Extreme cases of toxication may result in death although this is rare. Within Australia, only mild cases of poisoning have been reported, in Tasmania (Hallegraeff, 2002).

The symptoms of PSP include numbness, dizziness, nausea, tingling in the extremities, vomiting and diarrhoea in mild cases (within 30 minutes), to choking sensations, breathing difficulties and death from respiratory paralysis 2 – 24 hours after ingestion in severe cases (Hallegraeff, 1997).

*G. catenatum* is a major problem in SE Tasmania and has caused regular bloom events that have resulted in numerous harvest area closures. This species appears to have been introduced to Tasmania in 1973 and to Victoria later, probably in ballast water.

*G. catenatum* was first recorded at very low levels in Port Phillip Bay (PPB) on one occasion at the Dromana Aquaculture Fisheries Reserve (AFR) in 2002 and again at the Pinnace Channel AFR in 2014 and has not been recorded since, with no PST detected in biotoxin analysis.

In April 2022, *G. catenatum* was first recorded, at very low levels, in Western Port (WP) at the Flinders AFR.

In May 2024 a *G. catenatum* bloom resulted in a PSP biotoxin closure at the Flinders AFR with phytoplankton levels for *G. catenatum* remaining elevated (50 – 650 cells/L) for 8 weeks. PSP biotoxin levels remained just above the regulatory limit of 0.8 mg/kg for a period of 6 weeks resulting in an ongoing closure for 10 weeks. Product recall was undertaken by PrimeSafe and biotoxin signage warning of the risk of biotoxins in the vicinity of the AFR was instigated by DH.

**MANAGEMENT PROTOCOL**

The following management protocol has been designed to ensure that mussels harvested from PPB and WP are safe for human consumption, and that harvesting does not occur when the mussels are affected by biotoxins. The protocol is based on the following key factors:

- A number of species of *Gymnodinium* occur naturally in PPB and WP.
- Definitive identification of the various species of these genera may be difficult.
- *G. catenatum* has rarely been recorded from PPB, however, high levels of *G. catenatum* have recently been recorded in Western Port resulting in the instigation of a PST biotoxin closure in May 2024. This is the first known *G. catenatum* bloom to result in a PST biotoxin closure in Victoria.
- *G. catenatum* was introduced to SE Tasmania, probably in ballast water, and has become well established in the Huon and Derwent Estuaries. Shellfish farms have been adversely affected since 1986, with increasing numbers of biotoxin closures occurring since 2012 affecting shellfish farms as well as abalone and rock lobster fisheries.
- *G. catenatum* produces PSP with the potential to cause serious human illness.
- Extreme PSP intoxication is potentially lethal to human beings (3 children died in Mexico), although only mild cases have occurred in Tasmania attributable to *G. catenatum*.
- Routine phytoplankton sampling and biotoxin testing for PSP will continue at all VSQAP harvesting areas.

This management protocol has been devised for use within the VSQAP.

1. Phytoplankton samples are taken routinely each fortnight at all VSQAP harvesting areas,
2. Biotoxin samples are taken routinely each month and analysed for PSP.
3. If what is suspected to be *G. catenatum* is detected in a routine sample at an abundance of >1000 cells/L (1 cell/mL), a warning should be issued to the relevant growers and the sampling frequency reviewed with a view to increasing too weekly. This is important as previous history shows that the numbers of many phytoplankton can increase very rapidly.
4. Notify the laboratory performing the testing of the presence of this species.
5. If there is uncertainty concerning the identification as *G. catenatum*, PSP toxicity should be assumed until biotoxin levels are known.
6. Where *G. catenatum* is detected in numbers >5000cells/L, harvesting should be suspended pending on the results of tissue testing. The laboratory should be advised of the phytoplankton result and the urgency of the situation.
7. Where tissue is found to contain PSPs at a level exceeding 0.8 mg/kg (80 µg/100g) tissue (the regulatory limit), harvesting is to be suspended and is to remain suspended until two successive samples taken at least 7 days apart reveal toxin levels < 0.8 mg/kg tissue.

8. Where lower levels of toxin are detected during the growth phase of a bloom, harvesting should be suspended, and sampling frequency increased to monitor the development of the bloom.
9. Where toxin levels have exceeded the 0.8mg/kg tissue regulatory limit during a bloom, but the bloom is clearly degenerating, harvesting may be resumed once toxin levels remain less than 80μg/100g for two successive samples taken at least 7 days apart.
10. If *G. catenatum* is present, and/or low PSP levels are detected, the frequency of sampling should be reviewed and amended to ensure that it is adequate to detect changes in either phytoplankton or biotoxin levels in an effective and timely manner.
11. When harvesting is suspended during a toxic bloom, the sampling frequency may be reduced to the routine fortnightly monitoring program to save resources and costs. However, two "clear" biotoxin results ("clear" = < 0.8mg/kg tissue) taken at least 7 days apart are required before harvesting can resume.
12. Once *G. catenatum* and PSP toxins are undetectable, the routine sampling regime may be resumed.

**VSQAP Phytoplankton Abundance Threshold Levels (cells/L)**

| Phytoplankton Species | Toxin | Warning Issued to Growers | Tissue Testing | Harvest Suspension (Pending Toxin Analysis) | Harvest Resumption |
|---|---|---|---|---|---|
| *Gymnodinium catenatum* | PSP | 1000 | 1000 | 5000 | <0.8 mg/kg PSP for 2 successive samples over at least 7 days; phytoplankton abundance not rising. |
| **PSP REGULATORY LIMIT:** | 0.8 mg saxitoxin equivalents/kg tissue | | | | |

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## *Dinophysis acuminata* and *Dinophysis* spp.

**BACKGROUND**

*Dinophysis* are large, bag shaped dinoflagellates with well-developed sulcal lists and reduced epitheca (Hallegraeff, 2002). They are common in Australian waters but not often abundant.

Some *Dinophysis* species have been found to produce fat-soluble polyether compounds called Diarrhetic Shellfish Poisons (DSPs) that may accumulate in the shellfish that consume them. DSPs may cause illness in human consumers of contaminated shellfish.

The major symptoms of DSP poisoning are diarrhoea, vomiting, nausea and abdominal pain. There is also some evidence of tumour formation in the digestive system as a result of chronic exposure. Recovery occurs after about 3 days irrespective of medical treatment (Hallegraeff, 1997) and no human fatalities have been recorded.

Following the characterisation of okadaic acid (OA), okadaic acid derivatives were isolated from shellfish including the dinophysis toxins (DTX) incorporating the OA esters (DTX 3). Subsequently, other toxins have been included in this group despite their different chemical structures and modes of action. These include the pectenotoxins (PTX), pectenotoxin seco acids (PTX2-SA) and yessotoxins (YTX). Work in Australia by Burgess (2002), and in New Zealand for the Marlborough Sounds Shellfish Quality Program by MacKenzie (2002), has shown that PTX2-SA compounds are not toxic to humans. Consequently, for the purposes of the Victorian Marine Biotoxin Management Plan (VMBMP), PTX2–SAs are no longer regulated as a DSP toxin. Diarrheagenic effects have been demonstrated only for OA and DTX; PTX 1-4 have been found to cause liver damage and YTX damages cardiac muscle in mice (Hallegraeff, 1997).

The main bloom species in Port Phillip Bay (PPB) and Western Port (WP) appears to be *Dinophysis acuminata*. *Dinophysis acuminata* in Australian and New Zealand waters has not been found to produce significant amounts of OA or DTX, although it does produce significant amounts of PTX 2 that seems to be rapidly converted to non-toxic PTX2-SA in mussels. Mussels from PPB and WP have been tested for DSP revealing that PTX2-SA predominated with very small amounts of PTX2 and traces of OA. No DTX 1 or 2 was found. DTX 3 (OA esters) has not been tested to date but will be in future. Despite the consumption of large quantities of mussels from the aquaculture reserves in PPB and WP, there has never been a report of a case of diarrhetic shellfish poisoning.

The Commission of European Communities (CEC) has published draft regulations covering DSP toxins in shellfish where it is proposed that a limit of 16 µg/100g total DSP content including OA, DTXs and PTXs (CEC, 2001; Holland et al, 2002) be adopted. This is despite the fact that the EU expert working group on all fat-soluble marine algal toxins (2001) removed both the PTXs and YTXs from this group (Aune in MacKenzie, 2002). The later group suggested a limit for PTX of 15 □g/100g. PTX is still regarded as toxic and although currently included as a DSP for the VVMBMP, should be regulated separately. The FSANZ Food Standards Code regulatory limit for DSP is adopted for the VMBMP.

YTX and azaspiracids (AZA) which are included by some as DSPs but are chemically distinct, are not regulated in Australia. For the purposes of the VMBMP, these compounds are not considered to be members of the DSP group of toxins. The oral toxicity of the YTXs is questionable and neither YTXs or AZAs has been detected in Australian shellfish to date. Currently, these compounds are analysed using the LC-MS/MS method for DSP toxins. Within

the VMBMP, when DSP biotoxin testing is performed, quantitative testing is also carried out for YTX and AZA.

The species of Dinophysis known to be or likely to be recorded in PPB and WP are:

| | |
|---|---|
| *Dinophysis acuminata* | (produces principally PTX-2-SA in PPB) |
| *Dinophysis fortii* | (potential DSP producer in PPB) |
| *Dinophysis caudata* | (potential DSP producer but rare in Australia) |
| *Dinophysis acuta* | (potential DSP producer but rare in Australia) |
| *Dinophysis tripos* | (potential DSP producer, widespread in Australia but rare) |
| *Dinophysis hastata* | (potential DSP producer, widespread in Australia but rare) |

Since August 1999, only *Dinophysis acuminata* has occurred in numbers sufficient to initiate biotoxin sampling/analysis within harvesting areas in Port Phillip Bay (PPB) and Western Port.

Further information concerning the toxicity of *Dinophysis* spp in New Zealand may be found in Chang (2004) and Rhodes (2005).

**MANAGEMENT PROTOCOL**

This management protocol specifically relates to *Dinophysis acuminata* where abundance and biotoxin data has been collected over several years, but all other species are treated in the same way until additional information indicates otherwise. Most species do not generally occur in numbers sufficient to cause concern in Port Phillip Bay and Western Port. It is designed to ensure that mussels harvested from these waters are safe for human consumption and that harvesting does not occur when the mussels are affected by DSP toxins produced by various *Dinophysis* species. It is based on the following key factors:

- Various species of *Dinophysis* have been detected in Port Phillip Bay and Western Port, notably *D. acuminata.*
- A number of species are known to produce significant DSP levels in shellfish tissue at very low abundances.
- *Dinophysis acuminata* in Port Phillip Bay produces PTX2-SA but little OA & PTX and no DTX. OA esters (DTX 3) have not been tested for to date.
- PTX2-SA has been shown to be non-toxic to humans and is excluded as a DSP toxin.
- Due to its low abundance trigger, the numbers of *D. acuminata* can vary quickly between levels above and below the trigger level, making management difficult.
- The relationship between tissue DSP levels and *D. acuminata* numbers is poor.
- The symptoms of DSP are relatively minor and human deaths have never occurred.
- The trigger for *D. acuminata* is likely to be conservative and it has been found to be less toxic than some other Dinophysis such as *D. acuta* in New Zealand (Rhodes, 2005).
- Routine phytoplankton sampling for DSP producing phytoplankton will continue to occur at all Victorian Harvesting Areas.
- Analysis of mussel tissue for DSP will be undertaken if phytoplankton numbers exceed the specified phytoplankton trigger levels for biotoxin testing.

Tissue testing is based on phytoplankton triggers and is done routinely in the VMBMP. The following management protocol has been used successfully within the former VSQAP from August 1999 to December 2008 for the management of *Dinophysis acuminata* blooms. It has been adopted for the current Victorian Marine Biotoxin Management Plan (VMBMP) including the mussel growing areas in Port Phillip Bay and Western Port. It should be noted that the principal trigger for harvest suspension is the biotoxin level in shellfish tissue; phytoplankton abundance forms an additional, early warning trigger allowing precautionary closure pending biotoxin results.

- Phytoplankton monitoring and tissue testing for DSP toxins is carried out routinely at each Harvesting Area.
- It is recommended that more rapid sedimentation methods than gravity be used to concentrate samples for counting *Dinophysis* due to the necessity for a rapid turnaround time. It is also recommended that the concentration factor be X10 more than that usually used for counting algae due to the very low threshold value for *Dinophysis* and the necessity for greater accuracy at low abundance levels.
- If *Dinophysis acuminata* numbers exceed 1,000 cells/L, a tissue sample should be collected immediately, shucked and sent to the laboratory for DSP biotoxin analysis.
- If *Dinophysis acuminata* numbers exceed 2,000 cells/L, growers are to be notified and a voluntary suspension of harvesting implemented pending the biotoxin analysis.
- If the total DSP level in mussel tissue (OA, DTX and PTX but excluding PTX2-SA) exceeds the regulatory limit of 0.16 mg/kg (16 µg/100g) tissue, harvesting should be suspended, and sampling frequency increased. The latter is very important as the abundance of *Dinophysis acuminata* can vary from problem to non-problem levels within days.
- If the phytoplankton monitoring indicates that a *D. acuminata* bloom is developing (trend of increasing numbers), then the monitoring frequency should be increased and harvesting suspended if DSP levels exceed the Food Standards Code Regulatory Limit.
- Once harvesting has been suspended due to the presence of DSP in mussel tissue, harvesting may not be resumed until two successive "clear" biotoxin results are obtained at least 7 days apart. In this case, a clear biotoxin result means DSP levels less than 0.16 mg/kg (16 µg/100g) tissue.
- Because the abundance of *Dinophysis* spp. can rise and fall rapidly above and below the threshold levels for tissue testing and the suspension of harvesting, this may be a difficult situation to manage once closure has been initiated. This will also be complicated by the fact that abundance may not only vary rapidly with time, but also between sites.
- Other *Dinophysis* species should be dealt with using the same thresholds as for *D. acuminata*, until more information is gathered on their toxin production and toxicity.
- The abundance threshold values and biotoxin regulatory limits for *Dinophysis* spp. should be updated regularly as new information becomes available. This is particularly the case with DTX 3 and *Dinophysis acuminata*.

**VMBMP Phytoplankton Abundance Threshold Levels (cells/L)**

| Phytoplankton Species | Toxin | Warning Issued to Growers | Tissue Testing | Harvest Suspension (Pending Toxin Analysis) | Harvest Resumption Refer section 9.4 Re-opening criteria |
|---|---|---|---|---|---|
| *Dinophysis acuminata* | DSP | 1,000 | 1,000 | 2,000 | <0.16 mg/kg DSP for 2 successive samples taken not < 7 days apart; phytoplankton abundance not rising. |
| *Dinophysis caudata* | DSP | 1,000 | 1,000 | 2,000 | As Above |
| *Dinophysis acuta* | DSP | 500 | 500 | 1,000 | As above |
| *Dinophysis fortii* | ?DSP | 1,000 | 1,000 | 2,000 | As Above |
| *Dinophysis* spp. | ?DSP | 500 | 500 | 1,000 | As Above – precautionary only till further information available |
| *Prorocentrum lima* | ?DSP | 1,000 | 1,000 | 2,000 | <0.16 mg/kg DSP for 2 successive samples taken not < 7 days apart; phytoplankton abundance not rising. |
| **DSP REGULATORY LIMIT:** | 0.16 mg OA equivalents/kg tissue | | | | |

## *Prorocentrum lima*

*Prorocentrum lima* is another dinoflagellate which is oval in shape and bears a small anterior indentation. It has been recorded widely over southern Australia including in Port Phillip Bay, the Gippsland Lakes and Tasmania. It is a benthic or epibenthic species commonly found attached to seaweeds and shallowly in sand (Hallegraeff, 2002).

This species has been found to produce DSP overseas (specifically OA and DTX-1), including in New Zealand. However, its toxicity status in Australia is uncertain and a culture from WA was found to be non-toxic.

For the purposes of the VMBMP, it has been assumed that this species is a DSP producer similar to *Dinophysis acuminata*, and the abundance and biotoxin triggers utilised for *Dinophysis acuminata* have been adopted until more information becomes available. Hence the management protocol for *Dinophysis* should be utilised for this species as well.

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# *Karenia/Karlodinium* group

## *Karenia brevis*

## *Karenia* spp

## *Karlodinium micrum*

## *Gymnodinium impudicum*

Note: newly identified brevetoxin producing species *Karenia cristata* appeared in South Australian algal blooms in 2025. Please assess risk associated with this species as new information arises.

**BACKGROUND**

*Karenia* spp. are small, unarmoured dinoflagellate phytoplankton (golden-brown algae with a large nucleus). They have two flagella, one protruding from a horizontal girdle groove and the other from a vertical sulcus groove (Hallegraeff, 2002).

Many species belonging to this and related genera are common in Australian and New Zealand waters. When in bloom, a significant number of species within this group are fish killers, particularly where fish cannot avoid the blooms. Despite this, the vast majority don't appear to cause any adverse reactions in humans who consume shellfish from bloom areas in Australia or New Zealand.

It is stressed that the taxonomy of this group is poorly understood although this situation has improved recently with an increase in the research being carried out in New Zealand and Australia. Species of *Karenia* may be very difficult to identify definitively using light microscopy and expert assistance should be sought. Identification is best done with live material.

*Karenia brevis* in Florida, which is principally associated with fish kills, produces brevetoxins (BTX) that may cause non-fatal but unpleasant neurological symptoms in humans exposed to them by direct contact (*e.g.* swimming through blooms), inhalation (*e.g.* near fish kills or breaking waves containing blooms) or through the consumption of contaminated shellfish. For mild cases of intoxication, the symptoms include chills, headache, diarrhoea, muscle weakness, muscle and joint pain and vomiting 3 – 6 hours after exposure. In extreme cases, other symptoms may occur including paraesthesia, altered perception of hot and cold, difficulty breathing, double vision, and trouble talking and swallowing (Hallegraeff 1997, 2002). There have been no fatalities associated with NSP intoxication.

It is doubtful that *Karenia brevis* sensu stricto is present in Australia or New Zealand, but a group of morphologically similar species are. Approximately 180 cases of shellfish poisoning occurred in New Zealand in 1993. It was concluded that the symptoms experienced by these people after consuming shellfish, were most likely caused by an NSP toxin. However, it was also noted that other toxins were present apart from BTX including DSP, and that microbiological contamination may have played a role in the illness (Todd, 2000). It was later found that what was reported as *Karenia* cf *brevis* or *Gymnodinium* cf *mikimotoi* at that time may have contained four or more gymnodinioid species including what, in New Zealand, have been called *Karenia brevisulcata* and *K. sellifromis* as well as *Karenia mikimotoi*, *Karenia bidigitata* and *Gymnodinium aureolum*. At the 18th Marine Biotoxin Science Workshop (2001) in New Zealand, the consensus was that *K. mikimotoi* was the dominant organism present, although it is still unclear exactly what other species were present during the event, and which was/were responsible for the toxication.

*Karenia mikimotoi* is very common in Victorian waters and has been associated with fish kills. Like other species resembling *K. brevis*, it is a flattened species although much less so in extent. It has never been associated with human toxicity in Australia despite huge blooms of it including in the waters of shellfish harvesting areas, and large quantities of mussels have been consumed from areas where it was present.

There has only been a single shellfish poisoning incident attributed to NSP in Australia occurring in Gippsland, Victoria in 1994. This resulted from the consumption of wild stock mussels from the Tamboon Inlet. *Karenia* cf *brevis* was identified as the causative organism (Arnott, 1998; Todd, 2001). *Karenia* cf *brevis* has also been recorded in Port Phillip Bay (PPB) twice at the Clifton Springs Harvesting Area as part of the Victorian Shellfish Quality Assurance Program (VSQAP) phytoplankton monitoring, in numbers up to 32,000 cells/L (Arnott *et al* 1999), but there have been no reports of any type of shellfish poisoning over that period. Whether this was the same species as that at the Tamboon Inlet or another species resembling *K. brevis* is not known.

In the USA, *K. mikimotoi* produces only about one third as much BTX as *K. brevis* (Todd, 2002) and New Zealand isolates produce much lower levels than these. In Florida, shellfish harvesting is suspended when *K. brevis* numbers reach 5,000 cells/L (Hallegraeff, 2002).

Other species such as *Karenia selliformis* from New Zealand are known to be associated with fish kills and produce ichthyotoxins including gymnodimine. Gymnodimine is not a risk to human health and does not produce neurotoxic shellfish poisoning, although it can kill mice during bioassays. Although there is little evidence that these species are toxic to human consumers of shellfish, the ichthyotoxins they produce are not well understood and further local information should be gathered.

BTX testing by mouse bioassay is not routinely carried out in Australia currently but is in New Zealand. However, its interpretation is complex and can be complicated by the effects of other marine toxins and related compounds such as gymnodimine, Wellington Harbour Toxin and fatty acids naturally found in shellfish that may kill mice during bioassay but NOT indicate human toxicity from NSP (false +ve). The Cawthron Institute in New Zealand has developed more definitive methods LC/MS methods for NSP analysis that will improve the management of this biotoxin. and eventually replace the current ether mouse bioassay (Todd, 2002).

Work by the Cawthron Institute in New Zealand using this LC-MS analysis has failed to confirm BTX production in any of the Karenia species tested in that country, including *K. mikimotoi.*

At this time, within Port Phillip Bay and Western Port, few, if any, *Karenia* species, including flattened species similar to *K. brevis* and *K. mikimotoi*, seem to offer any marked potential for human toxicity from the consumption of shellfish. The risk appears slight but until more information is known, their presence should be monitored, and toxin testing performed when the threshold abundance levels are exceeded.

The potentially toxic *Karenia* known from southern Australia, and in particular from Port Phillip Bay and Western Port in Victoria include the following species, although New Zealand work shows that if a number of these species is toxic, toxicity is very low. Due to the uncertain state of the taxonomy of this group, some other fish kill species not yet found in Port Phillip Bay, Western Port or Australia have been listed. Out of this group of dinoflagellates, the *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001)* lists only *Karenia* cf *brevis* in its phytoplankton abundance trigger table. The more recent New Zealand

Phytoplankton Action Levels (May 2005) have been adopted use in for the VMBMP. The FSANZ Food Standards Code regulatory limit for NSP is adopted for the VMBMP.

| | |
|---|---|
| *Karenia brevis* | (NSP – BTX; Unlikely to be present in Aust.) |
| *Karenia* cf *brevis* | (?NSP; Flattened species like *K. brevis*; PPB, Gippsland, NZ) |
| *Karenia mikimotoi* | (low levels NSP; Fish kills; widespread incl. PPB, Gippsland Lakes. |
| *Karenia papilionacea* | (low levels NSP - New Zealand; fish kills) |
| *Karenia selliformis* | (NSP (gymnodimine) - New Zealand; fish kills) |
| *Karenia bidigitatum* | (low levels NSP - New Zealand; fish kills) |
| *Karenia digitata* | (fish kills; Hong Kong Harbour) |
| *Karenia* cf *longicanalis* | (fish kills, toxin?; Hong Kong, Tasmania (similar sp.) |
| *Karenia brevisulcata* | (fish kills, "Wellington Harbour" Toxin; Wellington Harbour only, NZ) (uncharacterised toxin? No BTX) |
| *Karlodinium micrum* | (fish kills, Australia, New Zealand) |
| *Gymnodiinium impudicum?? | on NZ list* |

**MANAGEMENT PROTOCOL**

The following management protocol relates to *Karenia brevis*, *Karenia cristata* the *Karenia* spp listed (including flattened Australian species similar to *K. brevis)*, *Karlodinium micrum* and *Gymnodinium impudicum*. It has been designed to ensure that mussels harvested from Port Phillip Bay and Western Port are safe for human consumption, and that harvesting does not occur when the mussels are affected by biotoxins. The protocols are based on the following key factors:

- A number of species of *Karenia* and related genera occur naturally in Port Phillip Bay and Western Port.
- Definitive identification of the various species of *Karenia* may be difficult.
- A form of *Karenia* in PPB has been identified as *Karenia* cf *brevis* (=*Gymnodinium* cf *breve*) in the past although the state of knowledge of the taxonomy of this group was incomplete at that time.
- Large blooms of *Karenia mikimotoi* have been recorded from Port Phillip Bay which were responsible for massive fish kills in 1950's.
- There is no evidence of an incident of NSP intoxication in Port Phillip Bay or Western Port despite blooms of *Gymnodinium/Karenia* occurring in the past, including *Karenia mikimotoi* and *Karenia* cf *brevis*.
- Based on New Zealand experiences, there may be a risk of NSP from *Karenia brevis* as Port Phillip Bay contains major ports and there is a real risk this species may become introduced to Australia. There is also a slight risk from *Karenia* cf *brevis* (other, flat *Karenia* species that resemble *K. brevis*) and the species listed above.
- In Florida, shellfish harvesting is banned when *K. brevis* abundance reaches 5,000 cells/L (Hallegraeff, 2002). *K. mikimotoi* produces only a third as much toxin in the USA as *K. brevis*, and even less in New Zealand. The *K. brevis* threshold levels recommended in the *Australian Victorian Marine Biotoxin Management Plan for*

*Shellfish Farming* of 1,000 cells/L for tissue testing and 5,000 cells/L for voluntary harvesting suspension and the issue of public health warnings seem very conservative in the light of recent advances in New Zealand. Consequently, the New Zealand Phytoplankton Action Levels for *Karenia* spp and related forms have been adopted for the VMBMP.

- There is no evidence that the other fish killing species can cause human intoxication from to the consumption of shellfish.
- Routine phytoplankton sampling for NSP producing phytoplankton will continue to occur at all Victorian Harvesting Areas.
- Analysis of mussel tissue for NSP will be undertaken if phytoplankton numbers exceed the specified phytoplankton trigger levels for biotoxin testing.

The following management protocol adopts the New Zealand NZFSA alert levels (May 2005). Expert assistance may be required to identify the relevant species. It has been used successfully within the VSQAP between August 1999 and December 2008 and has been adopted for the current Victorian Marine Biotoxin Management Plan (VMBMP) including the mussel harvesting areas in Port Phillip Bay and Western Port. It should be noted that the principal trigger for harvest suspension is the biotoxin level in shellfish tissue; phytoplankton abundance forms an additional, early warning trigger allowing precautionary closure pending biotoxin results and as a trigger for biotoxin testing.

1. If what is or suspected to be *Karenia brevis* is detected in a routine sample at an abundance of >1,000 cells/L (1 cell/mL), a warning should be issued to the relevant growers.
2. If the species is confirmed as *K. brevis*, a mussel tissue sample must also be collected and sent to the laboratory for biotoxin analysis as quickly as possible.
3. The sampling frequency should be reviewed to ensure it is adequate to detect a rapid increase in phytoplankton numbers.
4. If there is uncertainty concerning the identification as *Karenia brevis* NSP toxicity should be assumed until biotoxin levels are known.
5. Where *Karenia brevis* is detected in numbers >2,000cells/L, harvesting should be suspended pending the results of tissue testing. The laboratory should be advised of the phytoplankton result and the urgency of the situation.
6. Where any of the other *Karenia* species noted above (NOT *K. brevis*) are detected (there may be more than 1 species present) in numbers exceeding 100,000 cells/L, growers should be notified, and definitive identifications obtained.
7. If the numbers exceed 250,000 cells/L, a mussel tissue sample must also be collected and sent to the laboratory for biotoxin analysis.
8. If numbers rise above 300,000, harvesting should be suspended pending the results of biotoxin testing.
9. Where tissue is found to contain NSP (BTX) at a level exceeding 200 MU/kg (20 MU/100g) tissue (the regulatory limit) or 0.8BTX-2 eq mg/kg, harvesting is to be

suspended and is to remain suspended until two successive samples taken at least 7 days apart reveal toxin levels < 200 MU/kg or < 0.8BTX-2 eq mg/kg tissue.

10. Where lower levels of toxin are detected during the growth phase of a bloom, harvesting should be suspended, and sampling frequency increased to monitor the development of the bloom.
11. Where toxin levels have exceeded the 200 MU/kg or 0.8BTX-2 eq mg/kg tissue regulatory limit during a bloom, but the bloom is clearly degenerating, harvesting may be resumed once toxin levels remain less than 200 MU/kg or 0.8BTX-2 eq mg/kg for two successive samples taken at least 7 days apart.
12. If *Karenia brevis* or *Karenia* spp are present in numbers approaching their trigger levels and/or low NSP levels are detected, the phytoplankton and biotoxin sampling frequency should be revised to ensure adequate monitoring of any bloom that may develop.
13. When harvesting is suspended during a toxic bloom, the sampling frequency may be reduced to the routine fortnightly monitoring program to save resources and costs. However, two "clear" biotoxin results ("clear" = < 200 MU/kg or 0.8BTX-2 eq mg/kg tissue) at least 7 days apart are required before harvesting can resume.
14. Once *Karenia brevis*, or *Karenia* spp abundance is clearly less than the trigger values and NSP toxins are undetectable, the routine fortnightly sampling regime may be resumed.

**VMBMP Phytoplankton Abundance Threshold Levels (cells/L)**

| Phytoplankton Species | Toxin | Warning Issued to Growers | Tissue Testing | Harvest Suspension (Pending Toxin Analysis) | Harvest Resumption Refer section 9.4 Re-opening criteria |
|---|---|---|---|---|---|
| *Karenia brevis* (Not currently recorded in Australia) | NSP (BTX) | 1,000 | 2,000 | 5,000 | <200 MU/kg or 0.8BTX-2 eq mg/kg for two successive samples taken at least 7 days apart; phytoplankton abundance not rising |
| *Karenia mikimotoi, K. papilionacea, K. bidigitata, K. brevisulcata, K. selliformis* Flattened Australian species morphologically similar to *K. brevis* or *K. mikimotoi* *Karlodinium micrum, Gymnodinium impudicum* | ?NSP | 100,000 | 250,000 | 300,000 | As Above |
| **NSP REGULATORY LIMIT:** | 200 MU/kg or 0.8BTX-2 eq mg/kg tissue | | | | |

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## *Pseudo-nitzschia* spp.

**BACKGROUND**

*Pseudo-nitzschia* spp. are narrow, elongate diatoms that are difficult to identify to species level using light microscopy; generally, electron microscopy is required.

A number of species, notably *P. multiseries* and *P. australis*, have been found to produce the Amnesic Shellfish Poison (ASP) domoic acid that may be accumulated in the flesh of shellfish. ASPs may cause illness in people consuming contaminated shellfish such as mussels, oysters and scallops. It should be noted that toxicity within a species may be variable both with locality and time. Until definitive identification is obtained, it should be assumed that all the forms of *Pseudo-nitzschia* present are toxic.

A serious shellfish-poisoning outbreak in humans in Canada in 1987 resulted in memory loss in extreme cases of intoxication, and consequently, the syndrome was called Amnesic Shellfish Poisoning (ASP). The causative compound was found to be domoic acid. The symptoms of ASP are nausea, vomiting, diarrhoea and abdominal cramps after 3 – 5 hours. In extreme cases there may be a decreased reaction to deep pain, dizziness, hallucinations, confusion, short-term memory loss and seizures (Hallegraeff, 1997, 2002). A small number of deaths have occurred in Canada with immuno-depressed patients most at risk. There is evidence that the concentration of domoic acid in shellfish may be species dependant with scallops most at risk and mussels much less so. There are no documented cases of amnesic shellfish poisoning in Australia. Domoic acid has not been detected in Victorian mussels since the commencement of the Victorian Shellfish Quality Assurance Program (VSQAP) in 1987 but has been detected in scallops from Bass Strait (Arnott *et al*, 1999). However, in 2023/24 ASP at extremely low levels, was detected on two occasions in Western Port blue mussels.

The *Pseudo-nitzschia* species currently known from Port Phillip Bay (PPB) and Western Port (WP) in Victoria include the following. Several of these are new records for these areas being detected for the first time by previous VSQAP phytoplankton monitoring.

| | |
|---|---|
| *Pseudo-nitzschia multiseries* | (potentially toxic) |
| *Pseudo-nitzschia australis* | (potentially toxic) |
| *Pseudo-nitzschia pungens* | (non-toxic in PPB) |
| *Pseudo-nitzschia delicatissima* | (non-toxic in PPB) |
| *Pseudo-nitzschia pseudodelicatissima* | (non-toxic in PPB, mildly toxic in Derwent R.) |
| *Pseudo-nitzschia heimii* | (non-toxic) |
| *Pseudo-nitzschia fraudulenta* | (non-toxic) |

Additional information concerning Australian *Pseudo-nitzschia* and their toxicity may be found in Hallegraeff (1994) and Lapworth et al (2000), and for New Zealand in Chang (2004) and Rhodes (2005).

ASP (domoic acid) biotoxin sampling and analysis is carried out at Victorian Harvesting Areas monthly or when phytoplankton abundance triggers are exceeded.

**MANAGEMENT PROTOCOL**

The following management protocol has been designed to ensure that mussels harvested from Port Phillip Bay and Western Port are safe for human consumption, and that harvesting does not occur when the mussels are affected by ASP toxins. The protocol is based on the following key factors:

- *Pseudo-nitzschia* spp. are present as a component of the phytoplankton communities in Port Phillip Bay and Western Port for much of the year. They rarely form the dominant algal group within these communities (*i.e.* rarely > 50% of the total phytoplankton).
- The major blooms of this genus generally consist of *P. pseudodelicatissima*, *P. delicatissima* and *P. pungens* all of which have been found to be non-toxic in Port Phillip Bay however, *P.pungens* is difficult to separate from toxic species *P.multiseries* using light microscopy.
- Definitive identification of the various species of *Pseudo-nitzschia* may require electron microscopy, and they are therefore managed as a genus (group of species).
- *P. heimii* (non-toxic) and the potentially toxic species *P. australis* and *P. multiseries* have been detected as minor components of *Pseudo-nitzschia* blooms in Port Phillip Bay.
- There is a risk that the potentially toxic species *P. australis* and/or *P. multiseries* may become a major component of blooms.
- Due to the status of Port Phillip Bay and Western Port as harbours and the presence of a substantial number of foreign species in the former, probably introduced via ballast water, there is a danger that other toxic forms or species of *Pseudo-nitzschia* will be introduced.
- There is a risk that if environmental conditions alter in Port Phillip Bay, currently non-toxic *Pseudo-nitzschia* species may become toxic.
- Routine phytoplankton sampling for ASP producing phytoplankton will continue to occur at all Victorian Harvesting Areas.
- Analysis of mussel tissue for ASP will be undertaken monthly, and if phytoplankton numbers exceed the specified phytoplankton trigger levels for biotoxin testing.

The following management protocol has been used successfully within the VSQAP between August 1999 and December 2008 for the management of *Pseudo-nitzschia* blooms. It has been adopted for the current Victorian Marine Biotoxin Management Plan (VMBMP) including the mussel harvesting areas in Port Phillip Bay and Western Port. It should be noted that the principal trigger for harvest suspension is the biotoxin level in shellfish tissue; phytoplankton abundance forms an additional, early warning trigger allowing precautionary closure pending biotoxin results and as a trigger for biotoxin testing.

1. If *Pseudo-nitzschia* spp. are detected in numbers less than 100,000 cells/L (100 cells/mL), no further action but monitor numbers. Report presence to growers.
2. If *Pseudo-nitzschia* spp. (all species) are detected in numbers greater than 300,000 cells/L (300 cells/mL), or the numbers of *P. australis* plus *P. multiseries* exceeds

100,000 cells/L, institute ASP biotoxin testing as part of the routine sampling program. This analysis is additional to the current monitoring program.

3. If numbers (all species) exceed 500,000 cells/L, or the numbers of *P. australis* plus *P. multiseries* exceed 300,000, suspend harvesting pending the biotoxin analysis results.
4. If ASP is not detected, harvesting may be resumed immediately.
5. Continue ASP analysis as part of the fortnightly routine program at the affected harvesting areas until *Pseudo-nitzschia* spp. levels drop below 300,000 cells/L (300 cells/mL), or in the case of *P. australis* plus *P. multiseries*, below 100,000 cells/L.
6. If the main components of a bloom are found to be species known to be non-toxic, such as *P. pungens, P. delicatissima* and *P. pseudodelicatissima*, and ASP analysis is negative, continue to repeat step 5 until the bloom degenerates.
7. If any domoic acid is detected, it is recommended an industry warning be released and sampling frequency be increased to weekly.
8. If domoic acid is detected at levels > 20 mg domoic acid/kg tissue (the regulatory limit), harvesting should be suspended, and sampling frequency be amended with a view to increasing it to weekly.
9. Harvesting areas remain closed until domoic acid levels <20 mg/kg (<20µg/g) of tissue are found on two successive sampling occasions at least 7 days (one week) apart.
10. It should be noted that the risk from toxic *Pseudo-nitzschia* remains high for two weeks after the post bloom crash.
11. Once *Pseudo-nitzschia* levels drop below the relevant triggers and ASP is undetected in shellfish on two successive occasions at least 7 days apart, ASP sampling/analysis reverts to routine fortnightly phytoplankton analysis and monthly ASP analysis.
12. Re-evaluate the *Pseudo-nitzschia* trigger levels as more ASP testing is completed and related to *Pseudo-nitzschia* abundance over the period of the program.

### VMBMP Phytoplankton Abundance Threshold Levels (cells/L)

| Phytoplankton Species | Toxin | Definitive Identification & Warning to Growers | Tissue Testing | Harvest Suspension (Pending Toxin Analysis) | Harvest Resumption Refer section 9.4 Re-opening criteria |
|---|---|---|---|---|---|
| *Pseudo-nitzschia* spp. (*pseudodelicatissima* group) | ASP (domoic acid) | 100,000 | 500,000 | 500,000 | <20 mg/kg domoic acid for 2 successive samples over 14 days; phytoplankton abundance not rising. |
| *Pseudo-nitzschia australis* & *pungens*/*multiseries* (in *seriata* group) | ASP | 100,000 | 100,000 | 300,000 | As Above |
| **ASP REGULATORY LIMIT:** | 20 mg domoic acid /kg tissue | | | | |

**GENERAL**

It is stressed that this protocol is specifically designed for use within Port Phillip Bay and Western Port where an extensive record of the occurrence and toxicity of *Pseudo-nitzschia* exists extending from 1987 till the present. Where other regions are involved, due to the variability in the toxicity of the various forms of Pseudo-nitzschia, it would be prudent to follow the more conservative threshold levels proposed in the *Australian Victorian Marine Biotoxin Management Plan for Shellfish Farming (2001)* or those in other biotoxin management plans, which may be accessed through various websites.

This would also be the case if scallops were harvested rather than mussels, as some evidence exists suggesting that scallops are more likely to accumulate domoic acid than mussels. It is noted that domoic acid is known to bioaccumulate i.e. levels build up in organisms though food chains.

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## *Rhizosolenia amaralis*

**BACKGROUND**

*Rhizosolenia amaralis* is straight, cylindrical diatom, often found in chains.

Previously known as *Rhizosolenis cf. chunii,* the species was officially renamed *Rhizosolenia amaralis* in 2010. Blooms of this species can impart a bitter taste to mussels and other shellfish and render them unfit for human consumption. The chemical nature of the bitter taste is unknown, but the effect can persist for up to 7 months (Hallegraeff, 2002). In the 1987 Port Phillip Bay bloom, the digestive glands of exposed shellfish showed degeneration and significant mortality occurred 3 – 8 months after the bloom (Parry et al, 1989; Hallegraeff, 2002). Consequently, although posing no threat of toxicity to humans, the occurrence of blooms of this species constitutes a major threat to the mussel industry.

**MANAGEMENT PROTOCOL**

The following management protocol is designed to give growers warning of blooms of this species to facilitate management of their mussel resource. This potentially includes the transportation of mussels from impacted areas (not currently possible) to areas with lower numbers of *Rhizosolenia amaralis*, the suspension of harvesting and the withdrawal of affected mussels from the market. It is based on phytoplankton monitoring and the following information:

- *Rhizosolenia amaralis* belongs to the *Rhizosolenia 'imbricata'* group. Separation of *R.amaralis* from other species in this group requires detailed examination using transmission electron microscopy which is beyond the scope of most monitoring programs.
- *Rhizosolenia 'imbricata'* group is regularly detected within the Port Phillip Bay harvesting areas.
- While not all species within *Rhizosolenia 'imbricata'* group cause bitter taste, a conservative approach is taken given the potential to contain *R.amaralis*.
- In 1987, this species was responsible for making mussels unpalatable by imparting a bitter taste to them.
- Since then, other instances of this have been recorded by monitoring under the Victorian Shellfish Quality Assurance Program (VSQAP).
- The marketing of mussels with bitter taste imparted by *R. amaralis* would be deleterious to the aquaculture mussel industry.

This management protocol has been used successfully within the VSQAP from August 1999 to December 2008. It has been adopted for the current Victorian Marine Biotoxin Management Plan (VMBMP) including the mussel harvesting areas in Port Phillip Bay and Western Port.

1. Phytoplankton monitoring occurs routinely under the VMBMP.
2. Growers are to be kept updated regularly as to the abundance of *Rhizosolenia 'imbricata'* group at each harvesting area as each routine sampling event is carried out.
3. If *Rhizosolenia 'imbricata'* group is detected in numbers greater than 10,000 cells/L, a level one warning is to be issued to harvesters, advising that its abundance is rising.

4. If *Rhizosolenia 'imbricata group'* is detected in numbers greater than 20,000 cells/L, a level two warning is to be issued to harvesters that its abundance is approaching levels at which a bitter taste appears in mussels.
5. Additional sampling/monitoring may be undertaken by harvesters at any time.
6. Harvesters are to be informed once the threat has passed.

**VMBMP Phytoplankton Abundance Threshold Levels (cells/L)**

| Phytoplankton Species | Toxin | Warning Issued to Growers | Tissue Testing | Harvest Suspension (Pending Toxin Analysis) | Harvest Resumption Refer section 9.4 Re-opening criteria |
|---|---|---|---|---|---|
| *Rhizosolenia amaralis* (*imbricata* group) | Non-toxic Bitter Taste | 10,000 Level 1 Warning<br><br>20,000 Level 2 Warning | N/A | N/A | N/A |

Harvesting suspension is based on the presence of the bitter taste and is invoked voluntarily by harvesters as they see fit. The role of the VMBMP in relation to *R. amaralis* is to keep growers informed concerning the presence of this species.

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