# Brevetoxins – A Literature Review

## Report to the South Australian Brevetoxin Shellfish Working Group

**Stephen Pahl**

**SARDI Publication No. F2026/000098-1**
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Government of South Australia
Department of Primary Industries and Regions

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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*We ensure the land, sea, water and sky are healthy for future generations and strive to better understand their spiritual and cultural significance for Aboriginal people across the state.*

*In the spirit of reconciliation, our commitment is to build progressive and trusting relationships, to share knowledge and learn from each other.*

*We recognise and own a difficult past, and together we will walk forward.*

*We acknowledge the many Aboriginal people of this Country as the oldest continuous living culture in the world.*

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# Table of Contents

# List of Figures

# List of Tables

# Acknowledgements

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# Overview

Parts of South Australia are experiencing an unprecedented harmful algal bloom (HAB) that was initially reported to be dominated by the dinoflagellate *Karenia mikimotoi*, a species that has dominated previous blooms in the region, including a notable event in Coffin Bay in 2014. This current HAB has led to mass mortalities of a range of marine species and localized human health effects including respiratory, skin and eye irritation. The identification of *Karenia* sp. in environmental samples taken as part of the South Australian Shellfish Quality Assurance Program (SASQAP) initiated testing of commercial bivalves for brevetoxins. Brevetoxins were subsequently detected in some bivalve growing areas, and where necessary precautionary closures were implemented preventing the commercial harvest of bivalves from these locations.

This document was prepared for the South Australian Brevetoxin Shellfish Working Group to help respond to a series of questions. This included reviewing published literature on *K. mikimotoi* (the dinoflagellate species that was reported to be initially dominating the bloom), brevetoxins, and the identification of known or potential brevetoxin-producing species. The review has collated and summarised information from national and international food safety authorities, government bodies and research organisations and a number of original research articles.

*Karenia cristata* was subsequently identified by Murray *et al.* (2025) as occurring in the South Australian algal bloom as part of a mixed kareniacean assemblage and was confirmed to produce brevetoxins. This document provides a summary of existing data that is important to assist understanding of brevetoxins generally, including those that may be produced by *K. cristata*. Murray *et al.* (2025) also reported that *K. cristata* often dominated the South Australian algal bloom and has been identified in two other international locations, namely in South Africa (False Bay and Walker Bay) in the mid-1990s and a remote island off the coast of Newfoundland in Canada. The brevetoxin profile produced by *K. cristata* is different to that produced by *K. brevis*, in that only a subset of brevetoxins are produced – specifically BTX-2, BTX-3 and BTX-B5 (from the Type B backbone) with no BTX-1 (from the Type A backbone) detected. Other Kareniaceae taxa identified in water samples from the algal bloom were *K. longicanalis*, *K. mikimotoi*, *K. papilionacea*, as well as *Karlodinium veneficum* and *Takayama tasmanica* (Murray *et al.* 2025).

# Response to questions from the Brevetoxin Shellfish Working Group

The South Australian Brevetoxin Shellfish Working Group (BSWG) supplied the following questions on topics which they sought a greater level of clarity on and relevant information. High level responses to these questions are provided as a summary below. Further information is available in the body of the document.

**Background on brevetoxins**

Where and when have brevetoxin-producing algal blooms occurred and in which bivalve molluscs are affected (e.g. oysters/mussels/pipis)?

- Brevetoxins are primarily detected in seafood from coastal regions along the Gulf of Mexico/Florida, where *Karenia brevis* blooms reoccur near annually. Brevetoxins have also been detected in New Zealand and France, although in both instances the causative organism(s) that have produced the brevetoxins remain unknown.
- Most reported neurotoxic shellfish poisoning (NSP) cases in humans have been linked to the consumption of brevetoxin-contaminated recreationally harvested bivalves (notably mussels, clams, and oysters).
- The cause of the NSP outbreak in New Zealand in 1992-93 implicated contaminated New Zealand cockles (*Austrovenus stutchburyi*), Greenshell mussels (*Perna canaliculus*), and Pacific oysters (*Magallana gigas*).
- There was no information readily available in the literature regarding brevetoxins/brevetoxin-producing algal blooms effecting pipis, however, brevetoxins appear to be non-discriminate and can occur in organisms occupying all levels of the trophic chain. Following the identification of *Karenia sp*. in environmental samples taken as part of the South Australian Shellfish Quality Assurance Program (SASQAP) initiated testing of commercial bivalves for brevetoxins, and detected brevetoxins in some bivalve molluscs including within some Pipi (*Donax deltoides*) samples.

What is known about the detection of brevetoxins in other seafood (e.g. finfish, lobster, abalone, etc.)? - and provide any relevant context.

- Brevetoxins can transfer through the food web and be detected in a range of marine species from different trophic levels. Some gastropods (Whelks, Conch, and Horse conch) have been implicated in NSP events in Florida, USA. Whilst brevetoxins have been detected in a range of fish species, no evidence has been found in the literature that brevetoxins accumulate in fish muscle at concentrations that cause human illness.

What was the duration of the toxin presence in affected organisms?

- The has been a limited opportunity for researchers to examine brevetoxin uptake and elimination rates in field conditions aside from within the Gulf of Mexico/Florida. The reported rates can be highly variable between species. One study reported Eastern oysters became toxic 1-2 weeks after the onset of a *Karenia brevis* bloom. Other studies have reported variable durations, this is due to differing species being tested, differing conditions and different methods of analysis.

**Algal species known to produce brevetoxins**

Noting results to date have ruled out the presence of *Karenia brevis,* what other algal species could produce brevetoxins?

- The most well-known species producing brevetoxins is *Karenia brevis*. In 2025, Murray *et al.* (2025) identified that a rare and little known species, *K. cristata,* was present in the South Australian algal bloom and produces significant amounts of brevetoxins. *Karenia papilionacea* is also known to produce brevetoxins at very low levels. A range of other dinoflagellates belonging to the Kareniaceae family (including several other species from the *Karenia*, *Karlodinium* and *Takayama* genus) and some

raphidophytes (i.e. *Chattonella* spp., *Fibrocapsa japonica* and *Heterosigma akashiwo*) are known to produce brevetoxin-like compounds.

**Brevetoxins in shellfish**

How long has it taken for brevetoxins to leave the bivalve molluscs (oysters/mussels/pipis)?

- Elimination rates can be highly variable. Reported half-lives (duration in which the concentration decreases by half) is often between 3-10 weeks depending on metabolite and method of analysis.

In other regions internationally with brevetoxin occurrence, have brevetoxins reoccurred in the same areas (are we likely to expect to see this occur each year)?

- The most well documented region where brevetoxins occur is in the Gulf of Mexico/Florida, where *Karenia brevis* blooms occur near annually. The occurrence and magnitude of these blooms are unpredictable.
- Some microalgae can produce cysts as part of their life cycle. Cysts are dormant, resistant stages that allow the particular algae to survive un unfavourable environmental conditions. *Karenia* species are not known to produce long-term resting cysts, but can produce short-term “pellicle-cysts” that can extend a bloom.

**Farm management practices**

Could land based wet storage/depuration be used to facilitate toxin removal and return to safe food harvesting

- Depuration can be used to help remove a range of pathogens and/or toxins from bivalves and other shellfish. The efficacy can vary depending on the system, seafood species and level and nature of the contaminating pathogen and/or toxin. One international report stated that the depuration of lipid soluble biotoxins (such as brevetoxins) in shellfish is economically impractical. Additional research would be needed to confirm the viability of land based depuration to facilitate brevetoxin removal.

Does freezing/processing have an impact on the toxin?

- The concentration of brevetoxins in seafood is not reduced by rinsing, cleaning, cooking, or freezing.

# Introduction

Brevetoxins (BTXs) are a family of tasteless, odourless, heat- and acid-stable, lipid-soluble, cyclic polyether neurotoxins that are predominantly known to be produced by the bloom-forming dinoflagellate *Karenia brevis* and *K. cristata* (Murray *et al.* 2025). BTXs are toxic to fish, marine mammals, birds, and humans, but generally not to bivalve shellfish (Dickey *et al.* 2011). However, blooms of *K. brevis* and lysed *K. brevis* cells can impact bivalve larval survival (MacKenzie 2008). BTXs have been well-documented to accumulate in bivalves through their filter-feeding activities, and can also accumulate in fish and other marine organisms (EFSA Panel on Contaminants in the Food Chain 2010, Dickey *et al.* 2011, ANSES 2021).

In humans, exposure to aerosols containing BTXs can cause respiratory, skin and eye irritation. The ingestion of BTX-contaminated seafood can trigger neurotoxic shellfish poisoning (NSP). However, due to limited availability of quantitative data in both experimental animals and that related to human intoxications, the establishment of an oral acute reference dose has not been possible (EFSA Panel on Contaminants in the Food Chain 2010). The concentration of BTXs in seafood is not reduced by rinsing, cleaning, cooking, or freezing, and the toxins cannot be detected by taste or smell. The inability to easily detect toxins, other than through standard testing, and potential for elevated levels of BTXs in bivalves and potentially other marine organisms during and after blooms can put consumers at an increased risk for NSP. The risk of NSP is generally managed through monitoring seawater for known or suspected BTX-producing algae and where necessary testing of seafood for BTXs. The trophic transfer of BTX through the food web is a complex process, and a comprehensive understanding is currently lacking (Roth *et al.* 2008). However, a generalised scheme for the transfer of BTXs from *K. brevis* into the marine environment is shown in Figure 1. The BTX transfer from any other BTX-producer would likely be similar.

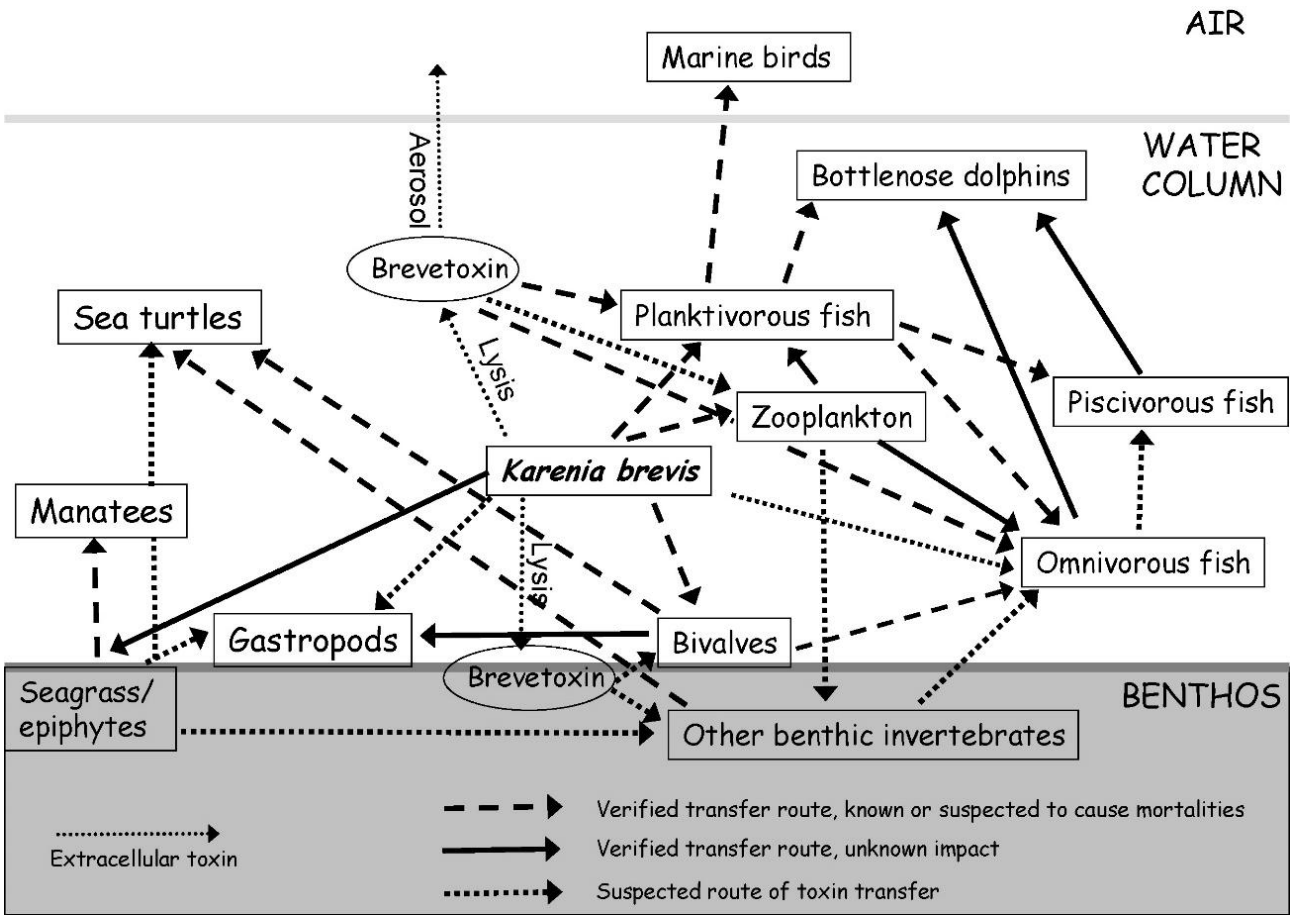



Figure 1 Schematic of the brevetoxin (BTX) transfer from *Karenia brevis* and impact in the marine environment. Reproduced from Landsberg *et al.* (2009).

Globally, a few hundred confirmed cases of human poisoning associated with the consumption of BTX-contaminated shellfish are documented in the literature (ANSES 2021, FSANZ 2021). UNESCO's Intergovernmental Oceanographic Commission provides a data portal on harmful algal events; and a summary of the geographical locations where *K. brevis* and NSP events have been reported to occur are shown in Figure 2.

Most reported NSP cases are linked to the consumption of recreationally harvested bivalves (notably mussels, clams, and oysters) (Watkins *et al.* 2008); however, some gastropods (whelks, conch, and horse conch) have also been implicated (Poli *et al.* 2000, Reich *et al.* 2015, Abraham *et al.* 2021). Consequently, educating the public on harmful algal bloom status and bloom movement, as well as warnings or advisories not to consume recreationally collected shellfish, are reported to be critical elements in the prevention of NSP illnesses (Watkins *et al.* 2008, Patel *et al.* 2020). Current guidelines in the United States of America do not limit the consumption of fillets from healthy finfish harvested during or after a *K. brevis* bloom. However, eating whole fish (as practiced by some individuals) may increase the risk of NSP, as the highest levels of BTXs in finfish are located in the liver and stomach contents (Watkins *et al.* 2008). BTXs may persist in finfish, particularly livers, for more than a year following bloom cessation (Watkins *et al.* 2008).

NSP symptoms generally start 30 minutes to three hours after ingestion and can last up to three days (EFSA Panel on Contaminants in the Food Chain 2010). NSP in humans is primarily characterised by the occurrence of gastrointestinal and neurological symptoms, generally including abdominal pain, nausea, vomiting, diarrhoea, paraesthesia (lips and extremities), dizziness, asthenia, partial paralysis of the limbs, speech impairment, loss of coordination and reversal of temperature sensation (Grattan *et al.* 2016, ANSES 2021, FSANZ 2021). NSP has also been reported to impact the cardiovascular system causing bradycardia and arterial hypotension (ANSES 2021) Whilst no human deaths associated with NSP have been documented internationally, symptoms can become quite serious. In some of the most severe cases, loss of consciousness, convulsions, tachycardia, and tachypnoea have been described, with some patients requiring ventilatory support (Watkins *et al.* 2008, ANSES 2021).


Figure 2 Global occurrence of *Karenia brevis* (blue squares) and NSP events (red circles). Data extracted from IOC-UNESCO Harmful Algae Information System (accessed 25 August 2025 from https://data.hais.ioc-unesco.org/).

*K. brevis* blooms (red tide events) reoccur near annually in the coastal regions along the Gulf of Mexico. These red tide events were recorded by Spanish explorers as early as the 17$^{th}$ century. Blooms of *K. brevis*

have occurred prior to the development of intensive agriculture, towns, industries and tourism, however, population growth and subsequent increase in nutrient run-off are considered to be the significant drivers for the recent increase in global HAB frequency, intensity and duration (Javaruski *et al.* 2022). Despite the almost annual occurrence of red tides in the eastern Gulf of Mexico, and except for one anecdotal and unconfirmed case involving consumption of whole fish, human intoxications from eating fish caught during or after *K. brevis* blooms have not been documented (Naar *et al.* 2007).

The only suspected NSP incident to be reported in Australia occurred after a number of people became sick after consumption of wildstock mussels from Tamboon Inlet, Gippsland Victoria in 1994. *Karenia brevis*-like species (*K*. cf. *brevis*) was identified in subsequent water samples, and wild mussels from the area had a toxin concentration of 27.5 mouse units (MU)[1] per 100g (Arnott 1998). No additional information is publicly available on this incident.

The largest recorded NSP outbreak internationally occurred in New Zealand in 1992-93, due to the consumption of contaminated New Zealand cockles (*Austrovenus stutchburyi*), Greenshell mussels (*Perna canaliculus*), and Pacific oysters (*Magallana gigas*) (Ishida *et al.* 1995, Morohashi *et al.* 1995, Ishida *et al.* 1996, Watkins *et al.* 2008). As a result, the entire coastline of New Zealand was closed to shellfish harvesting for several months, which was estimated to cost the shellfish industry $NZ100 million (Arnott 1998).

In November 2018, BTXs were detected for the first time in Europe in Blue Mussels (*Mytilus* spp.) from a lagoon in the Corsica region of France (Arnich *et al.* 2021). *Karenia brevis* was not detected as part of the monitoring program, and the causative algae had remained unknown.

# BTX Producers

The primary known producer of BTXs is the dinoflagellate *K. brevis* (formerly *Gymnodinium breve* and *Ptychodiscus brevis*). In 2025, *K. cristata* was identified as occurring in the South Australian algal bloom and confirmed for the first time to produce a significant amount of brevetoxins (Murray *et al.* 2025). A range of other microalgae, as listed in Table 1, have been reported or suspected to synthesize BTXs or BTX-like compounds. Determining which microalgae species produce BTXs at levels sufficient to cause human intoxication has been confounded by the lack of knowledge about the taxonomy of this group of algae, as well as differences in the methods of analysis used (McNabb *et al.* 2006, Victorian Fisheries Authority 2015).

It is challenging to distinguish many of these species under a light microscope. Microscopic-based monitoring requires a high level of taxonomic skill, takes considerable time, and accuracy can be highly variable among personnel (FAO 2004). This difficulty may contribute to confusion and uncertainty regarding the causative agents. Assessing the environmental threat posed by newly identified species of *Karenia* or other potential or suspected BTX producing algal species, is further complicated by the fact that they tend to co-occur with previously described species, and that there may be synergistic toxic effects caused by the presence of multiple species (Haywood *et al.* 2004, Haywood *et al.* 2007).

In 2006, McNabb *et al.* (2006) screened 34 known or suspected NSP-causative microalgae isolates (28 isolates had been sourced from New Zealand waters) using a liquid chromatography mass spectrometer technique. None of the New Zealand isolates were observed to produce BTXs, and only one (out of four) isolates of *K. brevis* (that had been recently sourced from the USA) produced BTXs. McNabb *et al.* (2006) highlighted the importance that when identifying BTX-producing microalgae any presumptive results from less-selective analytical methods should undergo adequate confirmatory tests.

[1] One mouse unit (MU) is the amount of crude toxin that will kill 50 percent of the test animals (20 g mice) in 930 minutes. The maximum level for neurotoxin shellfish poisons (i.e. brevetoxins) in bivalve molluscs in the Australia New Zealand Food Standards Code is 200 MU/kg.

Table 1 Microalgae known or suspected of producing brevetoxins and brevetoxin-like compounds.

| Species | BTX producer |
|---|---|
| *Chattonella marina* | • Suspected (ANSES 2021) (retention time of high -performance liquid chromatography-ultraviolet (HPLC-UV) peaks were compared to standards)<br>• BTX-like toxins (EFSA Panel on Contaminants in the Food Chain 2010) (see FAO 2004) |
| *Chattonella antiqua* | • Suspected (ANSES 2021) (retention time of HPLC-UV peaks were compared to standards)<br>• Potentially, non-toxic in New Zealand isolates (Hay *et al.* 2014)<br>• BTX-like toxins (EFSA Panel on Contaminants in the Food Chain 2010) (see FAO 2004) |
| *Fibrocapsa japonica* | • Suspected (ANSES 2021) (retention time of HPLC-UV peaks (BTX-like compounds called fibrocapsin) were compared to standards)<br>• Potentially, non-toxic in New Zealand isolates (Hay *et al.* 2014)<br>• BTX-like toxins (EFSA Panel on Contaminants in the Food Chain 2010) (see FAO 2004) |
| *Heterosigma akashiwo* | • Suspected (ANSES 2021) (retention time of HPLC-UV peaks were compared to standards)<br>• Potentially, non-toxic in New Zealand isolates (Hay *et al.* 2014)<br>• BTX-like toxins (EFSA Panel on Contaminants in the Food Chain 2010) (see FAO 2004) |
| *Gymnodinium aureolum* | • Potentially (detected by Enzyme-Linked Immunosorbent Assay (ELISA) test) (Hay *et al.* 2014) |
| *Gymnodinium impudicum* | • Potentially (detected by ELISA test) (Hay *et al.* 2014) |
| *Karenia bicuneiformis (formerly Karenia bidigitata)* | • Suspected (ANSES 2021) (one lab grown strain produced BTX; detected by ELISA test).<br>• Potentially (detected by ELISA test) (Hay *et al.* 2014)<br>• Yes (Hallegraff 2004) (was not substantiated in review) |
| *Karenia brevis* (formerly *Gymnodinium breve* and *Ptychodiscus brevis*) | • Yes (well documented) |
| *Karenia brevisculata* | • Potentially (Hay *et al.* 2014)<br>• Toxin or toxins produced have proved difficult to identify and there is still no defined structural solution (MacKenzie 2008) |
| *Karenia cristata* | • Yes (BTX-2, BTX-3 and BTX-B5 (Murray *et al.* 2025) |
| *Karenia concordia* | • Brevetoxin analogues (MacKenzie 2008)<br>• Potentially (Hay *et al.* 2014), |
| *Karenia mikimotoi* | • Suspected (ANSES 2021) (production of BTX mentioned by (Haywood *et al.* 2007) has never been confirmed).<br>• Potentially (detected by ELISA test) (Hay *et al.* 2014)<br>• Suspected causative agent in 1992/93 New Zealand outbreak (Dickey *et al.* 2011) – Refer to further discussion in the New Zealand Case study<br>• Confirmed to produce Gymnocin-A (Satake *et al.* 2002) |
| *Karenia papilionacea* | • Yes (per comm Shauna Murray and Kirsty Smith)<br>• Yes – PbTx-2 from mass spectrometry and nuclear magnetic resonance (NMR) experiments (Fowler *et al.* 2015)<br>• Confirmed producer of BTX-2 in laboratory conditions (ANSES 2021)<br>• Potentially (detected by ELISA test) (Hay *et al.* 2014)<br>• Yes (Hallegraff 2004) (was not substantiated in review) |
| *Karenia selliformis* | • Potentially (detected by ELISA test) (Hay *et al.* 2014)<br>• Yes (Hallegraff 2004) (was not substantiated in review) |

| | |
|---|---|
| *Karlodinium veneficum* (formerly *Karlodinium micrum*/ *Gymnodinium galatheanum*) | • Potentially (detected by ELISA test) (Hay *et al.* 2014) |
| *Takayama pulchella* | • Potentially – non-toxic in New Zealand isolates (Hay *et al.* 2014) |

*Karenia* species are known to synthesise a range of metabolites. Several species and their metabolites are reported below:

- *K. brevis* can produce brevetoxins, brevisin, brevisamide, brevenals, and tamulamides (ANSES 2021)
- *K. brevisulcata* can produce brevisulcenals and brevisulcatic acids (ANSES 2021)
- *K. mikimotoi* can produce gymnocins (ANSES 2021)
- *K. selliformis* can produce gymnodimines (ANSES 2021).
- *K. selliformis*, *K. longicanalis*, *K. papilionacea*, and *K. mikimotoi* were capable of producing gymnodimine-A (Cen *et al.* 2024)

# BTXs Compounds

In 2011, *K. brevis* was reported to produce at least nine BTX congeners grouped according to their Type A or Type B backbone structures (Type A: PbTx-1, PbTx-7, PbTx-10; Type B: PbTx-2, PbTx-3, PbTx-5, PbTx-6, PbTx-8, PbTx-9). PbTx-2 is the most abundant, whilst PbTx-1 is the most potent (Landsberg 2002, Dickey *et al.* 2011). The parent toxins (PbTx-1 and PbTx-2) are metabolised in bivalve molluscs to form multiple toxin analogues with varying potencies. The analogues can have a higher or lower potency than the parent toxin depending on the physiochemical properties. Consumers are therefore mainly exposed to the BTX-group metabolites rather than the parent toxins. Following the detection of BTXs in France in 2018, ANSES (2021) compiled a summary of the physicochemical properties of BTXs and other potentially toxic metabolites produced from *Karenia* spp. More than 70 BTX analogues and metabolites have been reported, of which 30 have known chemical structures (ANSES 2021). The molecular formula and monoisotopic mass of several BTX and BTX-like compounds produced by *Karenia* species are outlined in Table 2, whilst the chemical structures of several Type A and Type B BTXs are shown in Figure 3. Murray *et al.* (2025) identified that *K. cristata* produces three BTX analogues (BTX-2, BTX-3, BTX-B5). The BTX profile produced by *K. cristata* differs from that produced by *K. brevis*, with a notable absence of Type A backbone structure.

Table 2 Physicochemical properties of brevetoxins (BTXs) and other potentially toxic metabolites produced by *Karenia* species. Adapted from Hort *et al*. (2021) and Murray *et al*. (2025).

| Group | Metabolite | Synonyms | Molecular Formula | Monoisotopic Mass (Da) | *Karenia* species |
|---|---|---|---|---|---|
| A-type brevetoxins (BTX-A) | BTX-1 | PbTx-1; GB-1; T46, BTX-A | $C_{49}H_{70}O_{13}$ | 866.5 | *K. brevis* |
| | BTX-7 | PbTx-7; GB-7; Aldehyde-reduced PbTx-1 | $C_{49}H_{72}O_{13}$ | 868.5 | *K. brevis* |
| | BTX-10 [1] | / | $C_{49}H_{74}O_{13}$ | 870.5 | *K. brevis* |
| | Oxidized BTX-1 [2] | Oxidized PbTx-1; PbTx-1-carboxylic acid | $C_{49}H_{70}O_{14}$ | 882.5 | *K. brevis* |

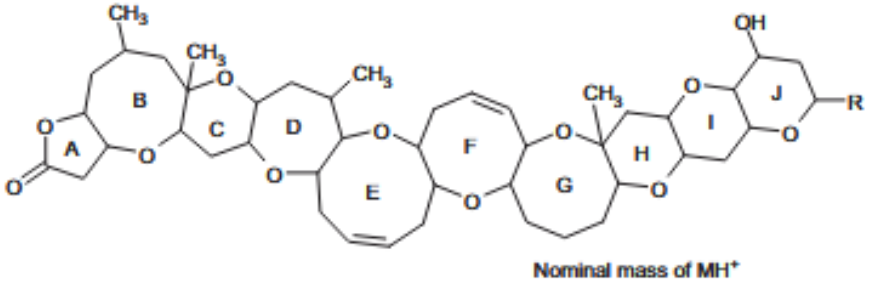
| Group | Metabolite | Synonyms | Molecular Formula | Monoisotopic Mass (Da) | *Karenia* species |
|---|---|---|---|---|---|
| | Open A-ring BTX-1 [2] | Open-ring PbTx-1 | $C_{49}H_{72}O_{14}$ | 884.5 | *K. brevis* |
| | Open A-ring, oxidized BTX-1 [2] | Open-ring, oxidized PbTx-1; PbTx-1-open ring-carboxylic acid | $C_{49}H_{72}O_{15}$ | 900.5 | *K. brevis* |
| | Open A-ring BTX-7 [2] | Open-ring PbTx-7 | $C_{49}H_{74}O_{14}$ | 886.5 | *K. brevis* |
| B-type brevetoxins (BTX-B) | BTX-2 | PbTx-2; T47; GB-2, T34, BTX-B | $C_{50}H_{70}O_{14}$ | 894.5 | *K. brevis, K. cristata* |
| | BTX-3 | PbTx-3; GB-3; T17; Dihydro-BTX-B; Aldehyde-reduced PbTx-2 | $C_{50}H_{72}O_{14}$ | 896.5 | *K. brevis, K. cristata* |
| | BTX-5 | PbTx-5; GB-5; acetylated PbTx-2 | $C_{52}H_{72}O_{15}$ | 936.5 | *K. brevis* |
| | BTX-6 | PbTx-6; GB-6; 27,28 epoxide of PbTx-2 | $C_{50}H_{70}O_{15}$ | 910.5 | *K. brevis* |
| | BTX-9 | PbTx-9; α-methylene reduced PbTx-3 | $C_{50}H_{74}O_{14}$ | 898.5 | *K. brevis* |
| | BTX-B5 | Oxidized BTX-2; Oxidized PbTx-2 | $C_{50}H_{70}O_{15}$ | 910.5 | *K. brevis, K. cristata* |
| | Open A-ring BTX-2 [2] | Open A-ring PbTx-2 | $C_{50}H_{72}O_{15}$ | 912.5 | *K. brevis* |
| | Open A-ring, oxidized BTX-2 [2] | Open A-ring, Oxidized PbTx-2; Open A-ring BTX-B5; PbTx-2-open ring-carboxylic acid | $C_{50}H_{72}O_{16}$ | 928.5 | *K. brevis* |
| | Open A-ring BTX-3 [2] | Open A-ring PbTx-3 | $C_{50}H_{74}O_{15}$ | 914.5 | *K. brevis* |
| Hemi-brevetoxins (Hemi-BTXs) | Hemi-BTX-A | GB-M | / | / | *K. brevis* |
| | Hemi-BTX-B | GB-N | $C_{28}H_{42}O_7$ | 490.3 | *K. brevis* |
| | Hemi-BTX-C | GB-4 | / | / | *K. brevis* |
| Brevenals | Brevenal | / | $C_{39}H_{60}O_8$ | 656.4 | *K. brevis* |
| | Dimethyl acetal brevenal | / | $C_{41}H_{66}O_9$ | 702.5 | *K. brevis* |
| Brevisamide | Brevisamide | / | $C_{18}H_{29}NO_4$ | 323.2 | *K. brevis* |
| Brevisin | Brevisin | / | $C_{39}H_{62}O_{11}$ | 706.4 | *K. brevis* |
| Tamulamides (Tams) | Tam-A | / | $C_{35}H_{45}NO_{10}$ | 639.3 | *K. brevis* |
| | Tam-B | / | $C_{34}H_{43}NO_{10}$ | 625.3 | *K. brevis* |
| Gymnocins | Gymnocin-A | / | $C_{55}H_{80}O_{18}$ | 1028.5 | *K. mikimotoi* |
| | Gymnocin-A carboxylic acid | / | $C_{55}H_{80}O_{19}$ | 1044.5 | *K. mikimotoi* |
| | Gymnocin-A2 | / | $C_{55}H_{80}O_{18}$ | 1028.5 | *K. mikimotoi* |
| | Gymnocin-B | / | $C_{62}H_{92}O_{20}$ | 1156.6 | *K. mikimotoi* |

| Group | Metabolite | Synonyms | Molecular Formula | Monoisotopic Mass (Da) | *Karenia* species |
|---|---|---|---|---|---|
| Gymnodimines (GYMs) | GYM-A | / | $C_{32}H_{45}NO_4$ | 509.4 | *K. mikimotoi, K. selliformis* |
| | GYM-B | / | $C_{32}H_{45}NO_5$ | 523.3 | *K. selliformis* |
| | GYM-C | GYM-B isomer | $C_{32}H_{45}NO_5$ | 523.3 | *K. selliformis* |
| Brevisucenals (KBTs) | KBT-A1 (sodium salt) | KBT-F sulfate ester | $C_{107}H_{159}O_{41}SNa$ | 2155.0 | *K. brevisulcata* |
| | KBT-A2 (sodium salt) | KBT-G sulfate ester | $C_{108}H_{161}O_{42}SNa$ | 2185.0 | *K. brevisulcata* |
| | KBT-F | / | $C_{107}H_{160}O_{38}$ | 2053.1 | *K. brevisulcata* |
| | KBT-G | / | $C_{108}H_{162}O_{39}$ | 2083.1 | *K. brevisulcata* |
| | KBT-H | / | $C_{107}H_{160}O_{39}$ | 2069.1 | *K. brevisulcata* |
| | KBT-I | / | $C_{108}H_{162}O_{40}$ | 2099.1 | *K. brevisulcata* |
| Brevisulcatic acids (BSXs) | BSX-1 | / | $C_{49}H_{72}O_{16}$ | 916.5 | *K. brevisulcata* |
| | BSX-2 | / | $C_{47}H_{68}O_{15}$ | 872,5 | *K. brevisulcata* |
| | BSX-3 [3] | / | / | 856.5 | *K. brevisulcata* |
| | BSX-4 | / | $C_{49}H_{70}O_{15}$ | 898.5 | *K. brevisulcata* |
| | BSX-5 | / | $C_{47}H_{66}O_{14}$ | 854.4 | *K. brevisulcata* |
| | BSX-6 [3] | Lactone derivative of BSX-3 | / | 838.5 | *K. brevisulcata* |
| | BSX-7 | / | $C_{47}H_{70}O_{14}$ | 858.5 | *K. brevisulcata* |

*[1] Synthesized toxin, unidentified in cultures to date, but postulated to occur naturally, based on structural correlation with BTX-9 and its presence in stationary cultures.*

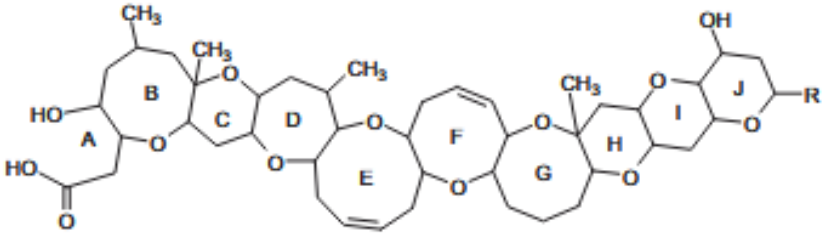
*[2] Analogues postulated after thorough study of LC-MS/MS fragmentations.*

*[3] Structure not elucidated.*



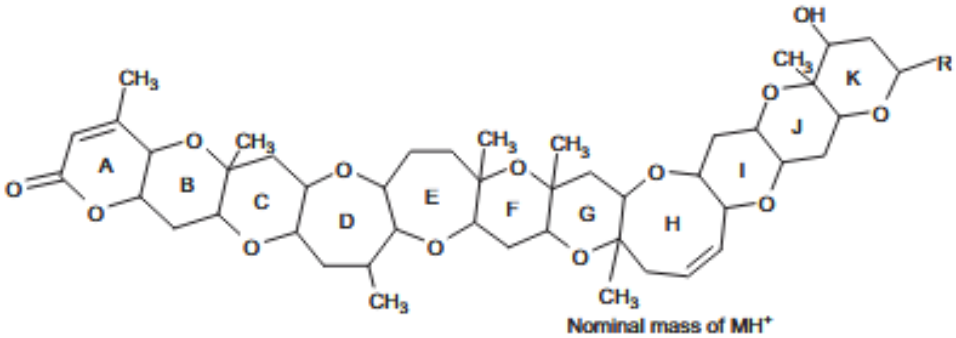
**Type A brevetoxins:**

| | | Nominal mass of $MH^+$ |
|---|---|---|
| PbTx-1 | R = $CH_2C(=CH_2)CHO$ | 867 |
| PbTx-7 | R = $CH_2C(=CH_2)CH_2OH$ | 869 |
| Oxidized PbTx-1 | R = $CH_2C(=CH_2)COOH$ | 883 |



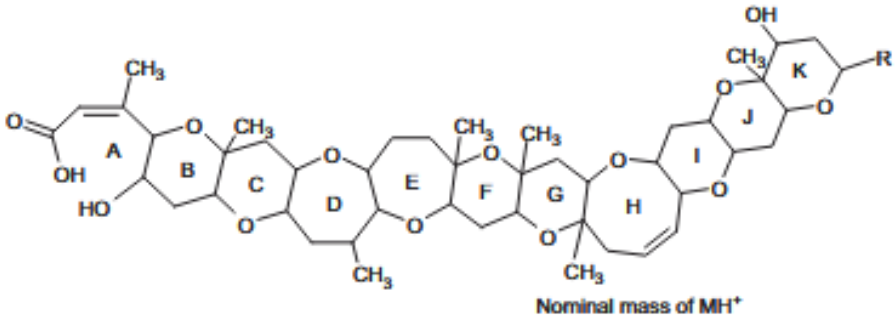
**Open A-ring Forms of Type A brevetoxins:**

| | | Nominal mass of $MH^+$ |
|---|---|---|
| Open A-ring PbTx-1 | R = $CH_2C(=CH_2)CHO$ | 885 |
| Open A-ring PbTx-7 | R = $CH_2C(=CH_2)CH_2OH$ | 887 |
| Open A-ring oxidized PbTx-1 | R = $CH_2C(=CH_2)COOH$ | 901 |



**Type B brevetoxins:**

| | | Nominal mass of $MH^+$ |
|---|---|---|
| PbTx-2 | R = $CH_2C(=CH_2)CHO$ | 895 |
| PbTx-3 | R = $CH_2C(=CH_2)CH_2OH$ | 897 |
| PbTx-9 | R = $CH_2C(=CH_3)CH_2OH$ | 899 |
| Oxidized PbTx-2 | R = $CH_2C(=CH_2)COOH$ | 911 |
| PbTx-2 adduct | putatively PbTx-2 methanol adduct | 927 |



**Open A-ring Forms of Type B brevetoxins:**

| | | Nominal mass of $MH^+$ |
|---|---|---|
| Open A-ring PbTx-2 | R = $CH_2C(=CH_2)CHO$ | 913 |
| Open A-ring PbTx-3 | R = $CH_2C(=CH_2)CH_2OH$ | 915 |
| Open A-ring oxidized PbTx-2 | R = $CH_2C(=CH_2)COOH$ | 929 |

Figure 3 Structural representation of type-A and type-B brevetoxins and their corresponding open A-ring hydrolysed and oxidised derivatives. Reproduced from Roth *et al*. (2008).

# Brevetoxin Regulatory/Trigger Limits

A residue toxicity of 20 MU per 100g (200 MU/kg) shellfish tissue has generally been adopted by regulators as a maximum permitted level. This is comparable to 0.8 mg BTX-2 equivalent/kg shellfish.

## Australia and New Zealand

The Australian Shellfish Quality Assurance Program's Operations Manual specifies that biotoxin risk management must consider all toxins listed in the Australia New Zealand Food Standards Code (Australian Shellfish Quality Assurance Advisory Committee 2024). Monitoring for neurotoxic shellfish toxins and marine biotoxins not listed in the Australia New Zealand Food Standards Code must occur when any evidence highlights a potential health risk.

Schedule 19 of the Australia New Zealand Food Standards Code[2] specifies a maximum level (ML) of 200 MU/kg for neurotoxic shellfish toxins in bivalve molluscs. The Australian Shellfish Quality Assurance Program's Operations Manual also provides guidance on when the Shellfish Control Authority can place harvest areas into closure and re-opening requirements.

Several Australian States also have phytoplankton action levels specified within their biotoxin management plans that if exceeded trigger management action, see Tables 3-5.

Table 3 NSW phytoplankton action levels for neurotoxic shellfish toxins (NST). Adapted from NSW Food Authority (2015).

| Phytoplankton species | Trigger value for flesh sampling (cells/L) | Issue public health warning (cells/L) |
|---|---|---|
| *Karenia* cf *brevis* | 1,000 | 5,000 |

Table 4 Tasmania phytoplankton action levels for neurotoxic shellfish toxins (NST). Adapted from ShellMAP (2019).

| Phytoplankton species | Alert level to contact ShellMAP immediately (cells/L) | Alert Level for ShellMAP to contact growers (cells/L) | Alert Level to initiate closure pending flesh testing results (cells/L) |
|---|---|---|---|
| *Karenia brevis* | 500 | 1,000 | 5,000 |
| *Karenia/Karlodinium/Gymnodinium* group[3] | 100,000 | 250,000 | 300,000 |

Table 5 Western Australian phytoplankton action levels for neurotoxic shellfish toxins (NST). Adapted from WA Department of Health (2020).

| Phytoplankton species | Alert level (cells/L)* | Alert level to initial flesh testing (cells/L) |
|---|---|---|
| *Karenia brevis* | 500 | 1,000 |
| *Karenia/Karlodinium/Gymnodinium group*[2] | 100,000 | 250,000 |
| * If exceedance, another sample is collected and analysed and if alert level is exceeded consider voluntary cessation. | | |

[2] Available at https://www.legislation.gov.au/F2015L00454/latest/text

[3] The *Karenia/Karlodinium/Gymnodium* group includes *Karenia bidigitata*, *Karenia brevisulcata*, *Karenia mikimotoi*, *Karenia papilionacea*, *Karenia selliformis*, *Karlodinium micrum* and *Gymnodinium impudicum*. If there is evidence of fish kills near the growing area, NST testing should be considered.

## New Zealand

The maximum permissible level for NSPs in bivalve molluscan shellfish is 0.8 mg BTX-2 equivalent/kg of edible portion (Ministry for Primary Industries 2024). New Zealand also has action levels for some harmful algae species (Table 6).

Table 6 Action levels in NZ for neurotoxic shellfish toxins (NST) in composite seawater sampling to trigger BMS flesh testing in New Zealand. Adapted from Ministry for Primary Industries (2024).

| Phytoplankton species | Cells per litre |
|---|---|
| *Karenia brevis* | 1,000 |
| *Karenia brevisculata* | 10,000 |
| *Karenia*/*Karlodinium*/*Gymnodinium* group[4] | 250,000 |

New Zealand's Ministry for Primary Industries also specifies that if paua (abalone), kina (sea urchin), crabs, rock lobster, eels and bivalves (processed for animal consumption) are harvested from water that is likely to be contaminated (such as with biotoxin), they must be managed in a way that minimises relevant risk factors (Ministry for Primary Industries 2025).

## Codex

*Standard for Live and Raw Bivalve Molluscs (CXS 292-2008)* specifies a ML of ≤200 MU or equivalent/kg molluscs flesh for the BTX group.

*Standard for Live Abalone and for Raw Fresh Chilled or Frozen Abalone for Direct Consumption or for Further Processing (CXS 312-2013)* species that where a risk exists, the marine biotoxins of concern shall be determined according to the methods specified in CXS 292-2008.

## USA

National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish (2023 Revision) specifies a guidance/action level or trigger/action levels for any BTX toxin found in shellfish meat at or above 20 MU per 100 g (0.8 mg BTX-2 equivalent/kg shellfish).

Haywood *et al.* (2007) noted that in the USA, the 5,000 *K. brevis* cells/L trigger level (to close commercial shellfish beds) was chosen because at this concentration, it takes approximately 24 hours for shellfish to accumulate enough toxin to pose a risk of NSP.

## Europe

There are currently no maximum limits on BTXs in shellfish in the European Union. However, following the detection of BTX in France, ANSES proposed a guidance value of 0.18 mg/kg (BTX-3 equivalents) of shellfish flesh (ANSES 2021). This guidance level is more than three times lower than the regulatory limits in Australia, New Zealand, and the USA[5].

ANSES (2021) recommends that if this guidance level is exceeded, then the following actions should be undertaken:

- Notify health professionals to improve the detection and reporting of any potential NSP cases.

[4] The *Karenia*/*Karlodinium*/*Gymnodinium* group includes *Karenia bidigitata*, *Karenia mikimotoi*, *Karenia papilionacea*, *Karenia selliformis*, *Karlodinium micrum* and *Gymnodinium impudicum.*

[5] ANSES (2021) reported that 20 MU/100g of shellfish flesh is equivalent to 0.8 mg BTX-2 equivalent/kg of shellfish flesh, which corresponds to 0.68 mg BTX-3 equivalents/kg

- Test for BTXs in other shellfish species produced in the affected area.
- Test for the potential presence of BTXs in shellfish in other production sites(s), including the open sea.
- Results should also be considered by the risk manager overseeing any nearby recreational fishing areas.

# Methods of Analysis

Methods of analysis are often grouped according to whether they are a biological or a chemical method. Each technique has its own advantages and limitations, and its application is often determined by the purpose of the test. Several techniques have been developed for the detection and/or quantification of BTXs. The relative specificity, sensitivity and overall efficacy of methods vary. The US NSSP (2023 Revision) lists the American Public Health Association (APHA) Mouse Bioassay (MBA) as the only Approved Method for NSP, whilst the MARBIONC Brevetoxin ELISA[6] method is an Approved Limited Use Method for NSP in oysters, Hard clams and Sunray Venus clams. The methods of analysis that are more commonly reported as being used for brevetoxin analysis in the literature are outlined below.

## Mouse Bioassay (MBA) – Biological Method

The MBA was developed in the 1960s and is used by several regulatory authorities. The protocol for the MBA as approved for use in the US NSSP (2023 Revision) is specified in Recommended Procedures for the Examination of Sea Water and Shellfish (American Public Health Association 1985). The MBA involves the evaluation of toxicity by intraperitoneal injection of a crude lipid extract of shellfish into mice. Results are expressed as mouse units (MU). One MU is the amount of crude toxic residue that, on average, will kill 50% of the test animals (20g mice) in 930 minutes.

Strengths:

- Provision of a measure of total toxicity based on the biological response of the animals to the toxin(s).
- Does not require complex analytical equipment.

Limitations:

- No specific information is provided on individual BTX-group toxins; however, some selectivity in BTX-group toxins may occur during the extraction process.
- Requires special animal facilities and expertise. Requires a relatively large amount of tissue extracts.
- Cannot be automated. Inherent variability in results between laboratories due to specific animal characteristics (strain, sex, age, weight, general state of health, diet stress).
- Has not been validated. It is laborious and time-consuming, which can result in delayed regulatory decisions.
- Use is considered undesirable in many countries for ethical reasons.

## Neuroblastoma cell assay (N2A) – Biological Method

A tissue culture technique using an established mouse neuroblastoma cell line (Neuro-2a). Several modifications to the original method have been reported.

[6] Developed by MARBIONC Development Group, LLC

Strengths:

- Sensitive and practical screening tool for detecting BTX-like activity.

Limitations:

- The method cannot distinguish between individual BTXs and can suffer from interferences (other sodium channel potentiating toxins).
- More sensitive to less polar BTX metabolites/analogues.

## Receptor binding assays (RBA) – Biological Method

The RBA is a functional bioassay developed for detecting a range of marine toxins. The assay can measure BTXs in homogenized shellfish tissue/extracts by the competitive displacement of tritium-labelled PbTx-3 from sodium channel binding sites in isolated rat brain synaptosomes. Incubation is carried out in a microplate format to minimise sample handling and to maximise sample throughput. The amount of BTX present (in PbTx-3 equivalents) in the sample is quantified by determining the amount of tritium-labelled PbTx-3 bound to the receptor, relative to the amount that binds in the absence of competing toxins.

Strengths:

- The method is relatively simple, sensitive and rapid.
- Multiple samples can be analysed simultaneously.
- The assay has been demonstrated to be helpful in assessing algal toxicity, for the purification of BTXs and for detecting BTXs in seafood.

Limitations:

- Lack of specificity – reacts to all toxins that bind to sodium channel receptors. The binding affinity of BTX metabolites/analogues is variable.
- Requires the use of radiolabelled compounds, requiring specially trained operators.

## ELISA (Enzyme-linked immunosorbent assay) – Biological Method

ELISA rapid test kits rely on the high specificity and sensitivity of antibodies to detect specific toxin analogues and to quantify toxin concentrations in solutions. Several commercially available ELISA kits are available and are often used as a screening tool, prior to further confirmatory analysis. It is important to consider whether ELISA kits are validated and for which tissue matrix. It is also important to understand the extent of any cross-reactivities.

Strengths:

- Rapid, high specificity and sensitivity.
- Ease of use.

Limitations:

- Do not provide any information on toxin profile.
- Cross-reactivity can occur.
- Not all kits on the market are validated.

## LC-MS (Liquid Chromatography Mass Spectrometry) – Chemical Method

An analytical chemistry technique that uses liquid chromatography to separate molecules from a sample followed by a mass spectrometer to identify and quantify the separated molecules based on their mass-

to-charge ratio. Some LC-MS instruments have tandem mass spectrometers (LC-MS/MS) which provide higher sensitivity, specificity and allow for structural characterisation of the molecules.

Strengths:

- High-sensitivity and high-specificity
- Can identify and quantify some individual BTX analogues

Limitations:

- Method requires expensive equipment, technical expertise, and reference standards[7] (many of which are not available or not readily available).
- The relative toxicological potency of individual BTX analogues is currently not well studied, impacting risk assessments and determining overall toxicity of a sample.

### Summary of methods of analysis

Dickey *et al.* (2004) reported findings from an interlaboratory comparative study on the performance of five methods (MBA, N2A, RBA, competitive ELISA and standard LC-MS). At the time they concluded that the competitive ELISA and one of the RBA assays "compared most favourably" and were "good alternatives" to the MBA for the determination of BTXs in shellfish. Dickey *et al.* (2004) also stated that the N2A was "extremely sensitive" but exhibited data variation and the LC-MS performed "as well as ELISA" but lacked metabolite standards and uniform instrument parameters.

In a latter study Plakas *et al.* (2008) monitored the concentration of BTXs in Eastern oysters (*Crassostrea virginica*) by several different analytical methods. Plakas *et al.* (2008) reported that ELISA and standard LC-MS methods provided rapid BTX screening and confirmation, respectively, and when used in combination were "excellent alternatives to the mouse bioassay for assessing oyster toxicity". Fang and Qiu (2023) considered the strengths and weaknesses of a variety of BTX measuring techniques and reported that LC-MS/MS methods are currently the "preferred method" as they have the advantages of "high selectivity and sensitivity"

In mid-1993 New Zealand established weekly nationwide toxic phytoplankton and shellfish biotoxin monitoring (by mouse bioassay), and in 2002 phytoplankton surveillance was combined with LC-MS/MS for routine monitoring (MacKenzie 2008).

## BTX accumulation and elimination studies in oysters

There has been limited opportunity to examine BTX uptake and elimination rates under field conditions aside from the within the Gulf of Mexico/Florida. In 2021, ANSES reported that there are few quantitative data on BTX accumulation and elimination rates in bivalves. Of the known studies, the kinetics of BTX elimination in bivalves have frequently been reported to be dependent on the bivalve species and elimination rates are highly variable, even under controlled conditions. Viviani (1992) stated that depuration of lipid soluble (lipophilic) biotoxins (such as BTXs) is economically impracticable due to the lengthy procedures required.

The following is a chronological of known documented accumulation and/or elimination studies or reviews.

### Shumway *et al.* (1990) and Shumway (1990)

These review articles on HABs report a retention time of 2-6 weeks for BTXs in *Crassostrea virginica* exposed to *K. brevis*. Both reviews cited findings from Morton and Burklew (1969)[8].

[7] Whilst a few certified standards are commercially available, the analysis of shellfish and fish for BTXs is often complicated by the availability of certified standards for other BTX-analogues and reference materials.

[8] The author has not been able to obtain a copy of the publication by Morton and Burklew (1969) to review.

## Dickey *et al.* (1999)

Field-exposed Eastern oysters (*Crassostrea virginica*) were sampled from 21 stations during and after a *K. brevis* bloom that impacted commercial shellfish reefs along the Mississippi Sound (Gulf Coast of the United States). Oyster tissue was analysed by mouse bioassay, cytotoxicity and receptor binding assays. BTX concentrations were converted into PbTx-3 equivalence for comparative assessment. Time-based results from two sampling sites were provided in the publication, reproduced here in Figure 4. BTX concentrations in the oyster tissue generally decreased following bloom dissipation, but the concentrations fluctuated throughout the study. There was also a notable increase in the BTX concentrations around 50 days after bloom dissipation, however, no discussion or rationale was provided on the cause or potential causes of these fluctuations. The authors concluded that the oysters were toxic by mouse bioassay for up to 75 days after dissipation of a *K. brevis* bloom.

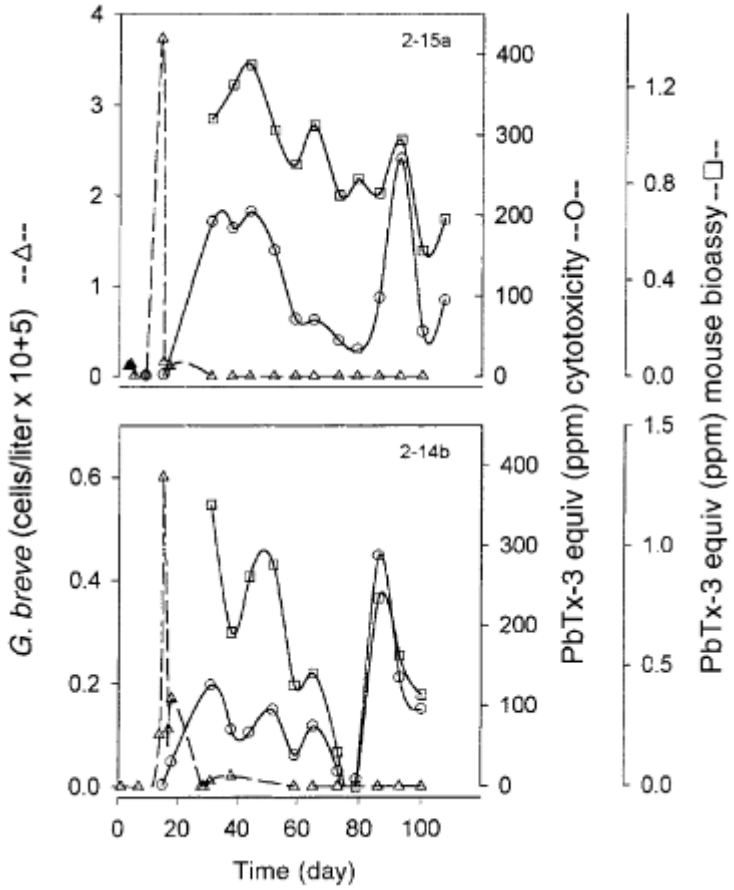



Figure 4 Time profiles of brevetoxins (PbTx-3 equivalence) concentration in Eastern oysters and bloom concentration at two sampling locations along the Mississippi Sound, USA. Reproduced from Dickey *et al*. (1999).

## Plakas *et al.* (2002)

Reported findings from a controlled elimination study when Eastern oysters (*C. virginica*) were exposed to isolated brevetoxins (PbTx-2 and PbTx-3) or *K. brevis* cells (from culture). The focus of the study was examining metabolite profiles in the oysters following exposure. The authors reported that “waterborne PbTx-3 was rapidly accumulated, but not metabolised, in the oysters, and was largely eliminated within 2 weeks after exposure. In contrast, PbTx-2 was accumulated and rapidly metabolised”. Metabolites of PbTx-2 included PbTx-3 and a cysteine conjugate. A second cysteine conjugate was detected but the authors speculated it to be an artefact of sample preparation and storage as its concentration did not

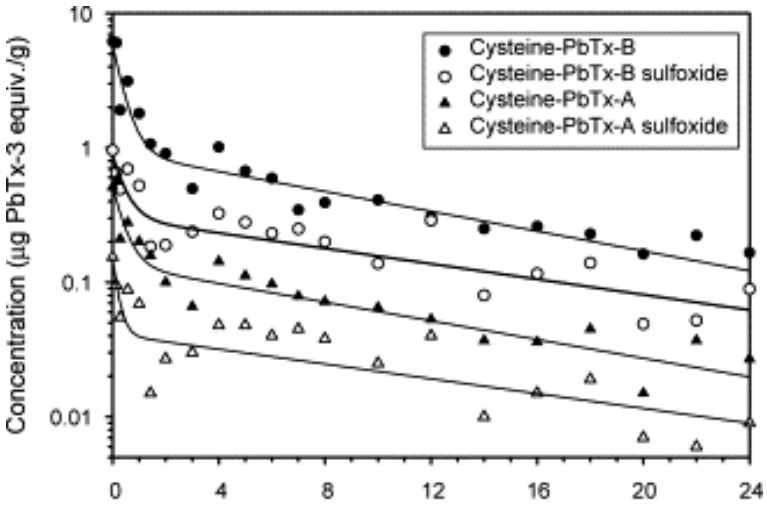
Morton, R.A and Burklew, M.A (1969). Florida shellfish toxicity following blooms of the dinoflagellate, *Gymnodinium breve*. Florida Department of Natural Resources Marine Research Laboratory. St Petersburg, Florida.

significantly change over the study. The concentration of PbTx-3 (metabolite) in PbTx-2 exposed oysters was highest immediately after exposure and decreased at a similar rate to parent PbTx-3 in PbTx-3 exposed oysters. The cysteine-PbTx conjugate persisted for 8 weeks after exposure.

## **Plakas *et al.* (2004)**

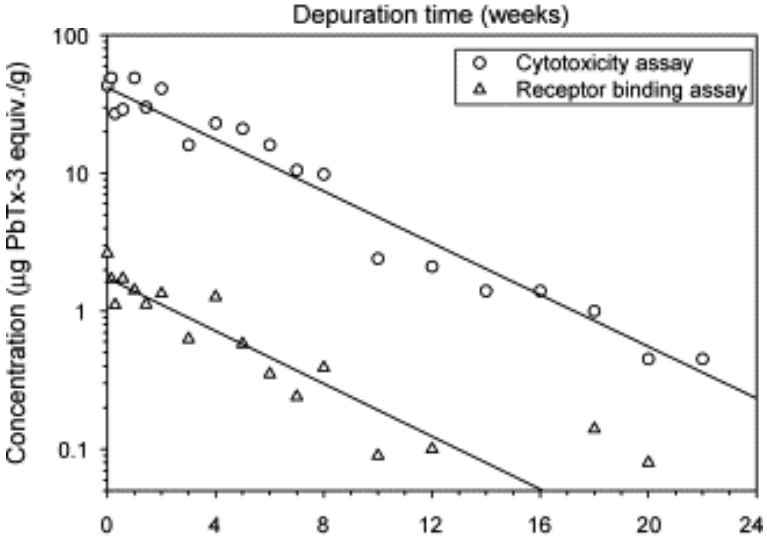
Reported findings from a controlled laboratory uptake and elimination study using *K. brevis* cultures and the Eastern oyster (*C. virginica*) with a two-day exposure period to approximately 62 million *K. brevis* cells/oyster (cleared within a few hours after addition). Oysters were subsequently placed in flowthrough tanks supplied with ambient seawater (no other details provided, e.g. natural plankton level, temperature, or salinity, were not reported). Several different methods of analysis were undertaken and the concentration of BTXs (BTX-3 equivalents/100g) during the depuration phase are shown in Figure 5. Summary of results:

- PbTx-3 and PbTx-9 (metabolic reduction of PbTx-2) were eliminated within 1-3 weeks.
- PbTx-7 and PbTx-10 (reduced forms of PbTx-1) were not detected.
- Cysteine conjugates of PbTx-1 and PbTx-2 and their sulfoxides were detectable for up to 6 months.



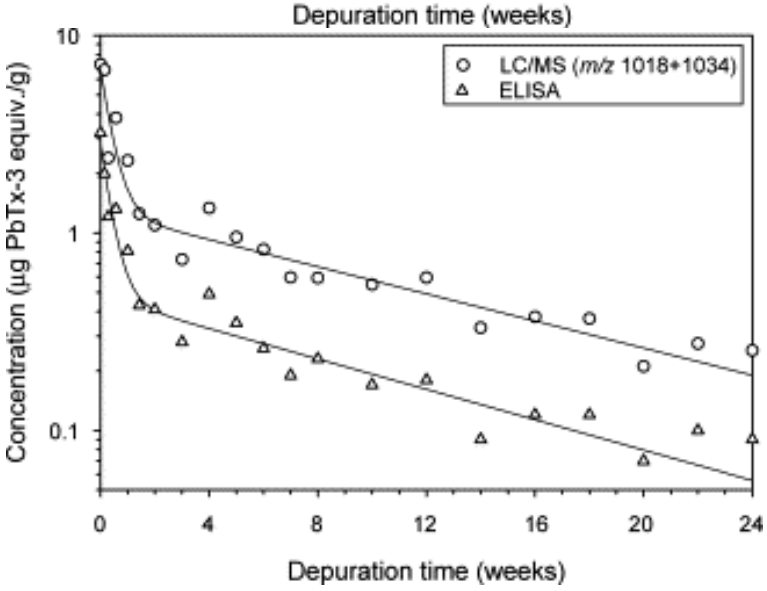

Elimination half-lives were reported in Dickey *et al.* (2011)

- Cysteine- PbTx-B – 8.2 weeks;
- Cysteine-PbTx-B sulphoxide – 10.5 weeks;
- Cysteine-PbTx-A – 8.7 weeks;
- Cysteine-PbTx-A sulphoxide – 10.9 weeks.




Elimination half-lives were reported in Dickey *et al.* (2011)

- 3 weeks for RBA
- 3.2 weeks for cytotoxicity assay




Elimination half-lives were reported in Dickey *et al.* (2011)

- 7.8 weeks by ELISA
- 7.9 weeks by LC-MS

Figure 5 Elimination of BTX metabolites in the Eastern oysters (*C. virginica*) after exposure to *K. brevis*. Reproduced from Plakas *et al*. (2004).

## Plakas *et al.* (2008)

Examined the uptake and elimination of BTXs in Eastern oysters (*C. virginica*) exposed to recurring *K. brevis* blooms over a 3-year period. Time-based BTX concentrations are shown in Figure 6. Authors reported that the oysters were "toxic at ≥20 MU/100 g by mouse bioassay generally at 1–2 weeks after a

bloom onset, when cell densities exceeded 5,000 cells/L". Brevetoxins were measurable by *in vitro* assays and LC-MS for several months (up to 8 months) after bloom dissipation.

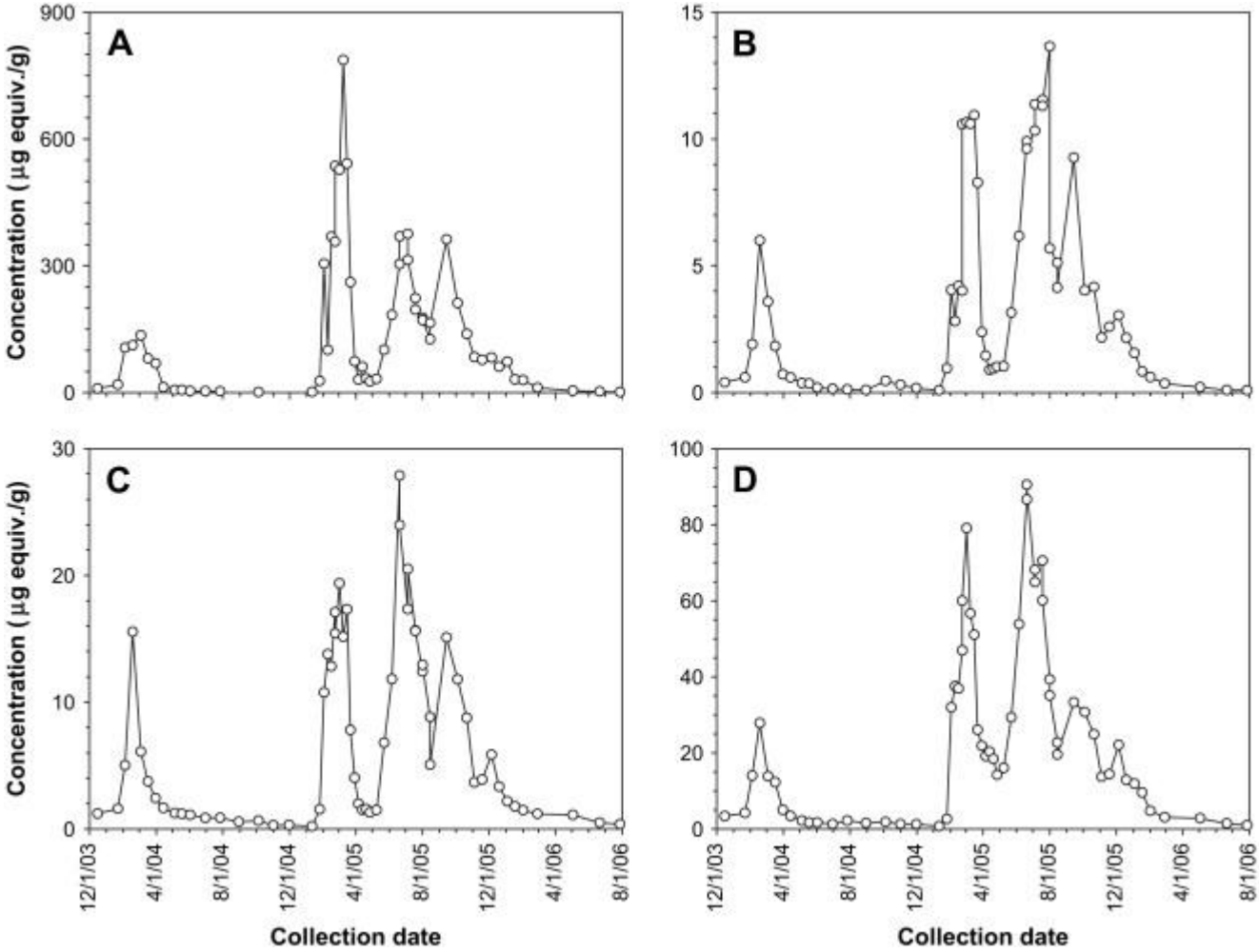



Figure 6 Time profiles of brevetoxin concentrations in Eastern oysters (*C. virginica*). Reproduced from Plakas *et al*. (2008), where (A) is cytotoxicity assay, (B) is receptor binding assay, (C) is ELISA assay and (D) is LC-MS.

## Watkins *et al.* (2008)

The review article reported that the depuration time of brevetoxins in shellfish varies but is typically within two to eight weeks. It acknowledged that there are reports of brevetoxins being retained much longer (nearly one year post bloom).

## Dickey *et al.* (2011)

Reported that Eastern oysters (*C. virginica*) accumulate toxins in less than four hours in the presence of 5,000 *K. brevis* cells/mL and eliminate 60% of the accumulated toxins in 36 hours.

## McFarland *et al.* (2015)

Assessed the rate of BTX uptake and elimination in Green mussels (*Perna viridis*) (average length 74.3±7.3mm) during a naturally occurring *K. brevis* blooms in southwest Florida, USA over 2011/12 and 2012/13. BTX concentrations were analysed via ELISA kits. The concentration of BTXs and BTX uptake and elimination rates were compared to levels detected in the Eastern oyster (*C. virginica*) that were sampled during the same period. The concentration of BTXs detected in Green mussels and the concentration of *K. brevis* from two sampling regions are reported in Figures 7 and 8, respectively. The mussels accumulated high levels of BTXs during the bloom. The Green mussels also retained higher BTX concentrations in their tissues post bloom and remained above the regulatory limit for 4–5 months. This

was significantly longer than the depuration time of 2–8 weeks for Eastern oysters and a native Hard clam (*Mercenaria mercenaria*).

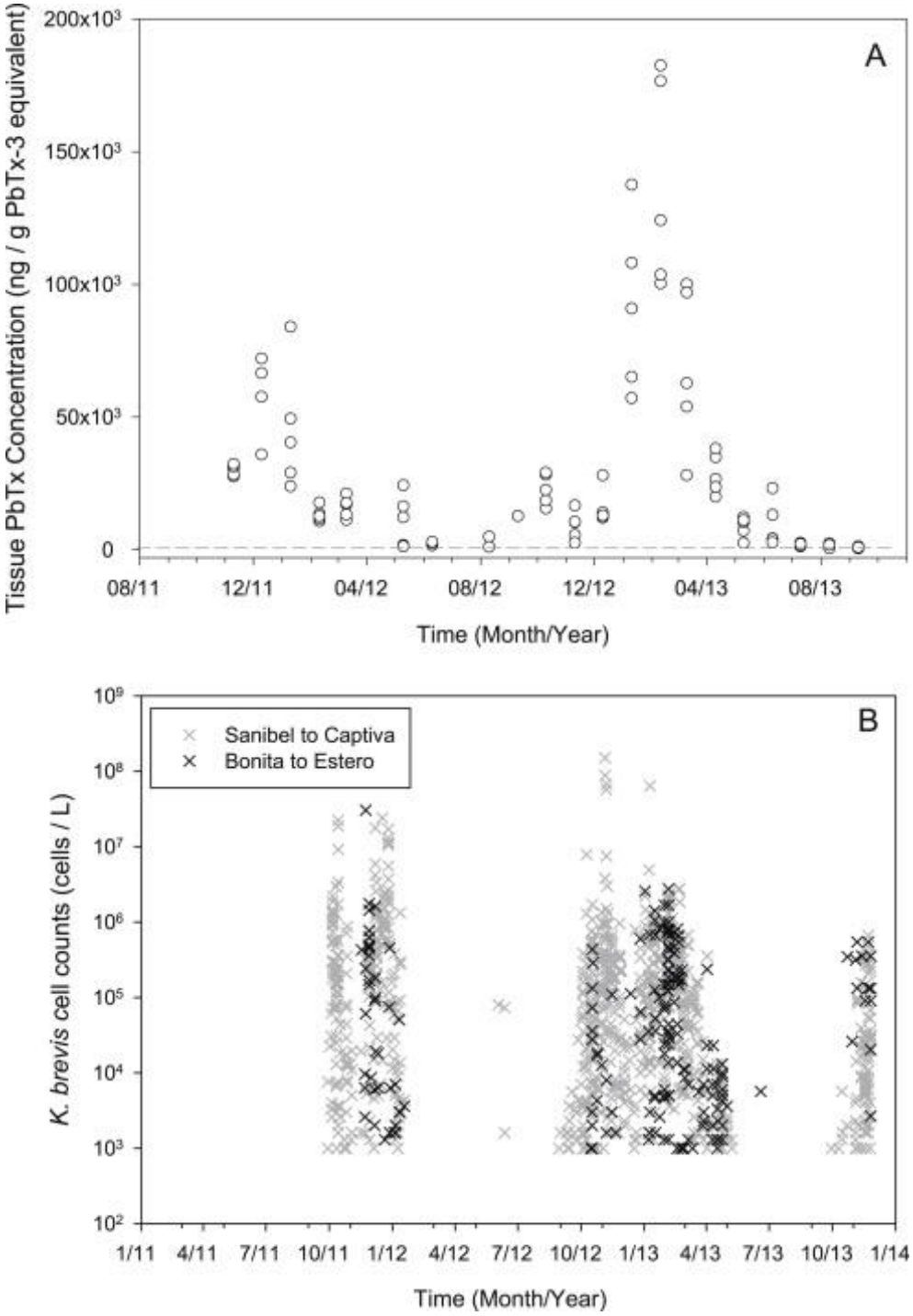



Figure 7 (A) Brevetoxin concentration in individual Green mussels (*P. viridis*) plotted over time. Dashed line indicates the regulatory limit (800 ng $g^{-1}$ PbTx-3 equivalent). (B) *Karenia brevis* cell counts (log scale) by location over time. Reproduced from McFarland *et al*. (2015).

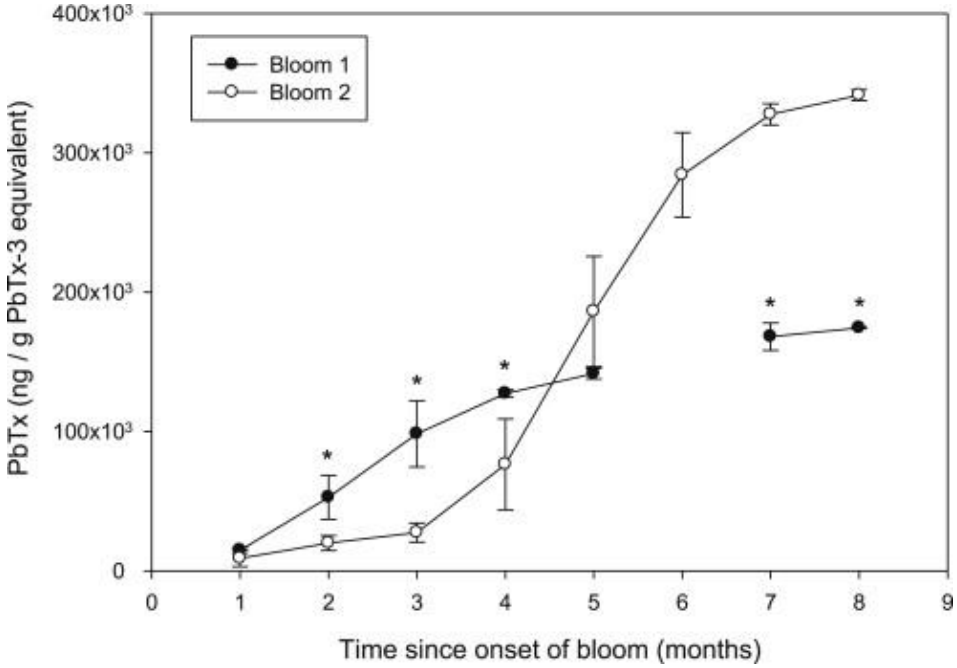



Figure 8 Concentration of PbTx-3 equivalent in Green mussels (*P. viridis*) tissue. Reproduced from McFarland *et al*. (2015).

## Griffith *et al.* (2013)

Individual Hard clams (*Mercenaria mercenaria*) and Eastern oysters (*C. virginica*) were exposed to *K. brevis* at a bloom concentration of $5x10^5$ cells·$L^{-1}$ for eight days and then transferred to filtered water for depuration. Experiment was undertaken under controlled laboratory conditions. BTXs were measured via ELISA.

Eastern oysters and Hard clams accumulated BTXs within three days. After five days the Hard clams had a BTX concentration of 1.0 mg/kg, whereas the Eastern oysters had a BTX concentration of 2.0 mg/kg. BTX concentrations were below regulatory levels (0.8 mg BTX-3 equivalent/kg) after two weeks of depuration. However, low levels of BTXs were present in shellfish tissues after 4 months. Toxins (or their metabolites) remained detectable until the end of the experiment in the clams (0.058 ± 0.239 mg/kg at 139 days) and in the last remaining oysters (0.117 mg/kg at 82 days). The time-based concentration of BTXs in the oysters and clams are shown in Figure 9. Low levels of BTXs (0.06 ± 0.037 mg BTX-3 equivalent/kg) were also detected in some of the control oysters throughout the experiment.

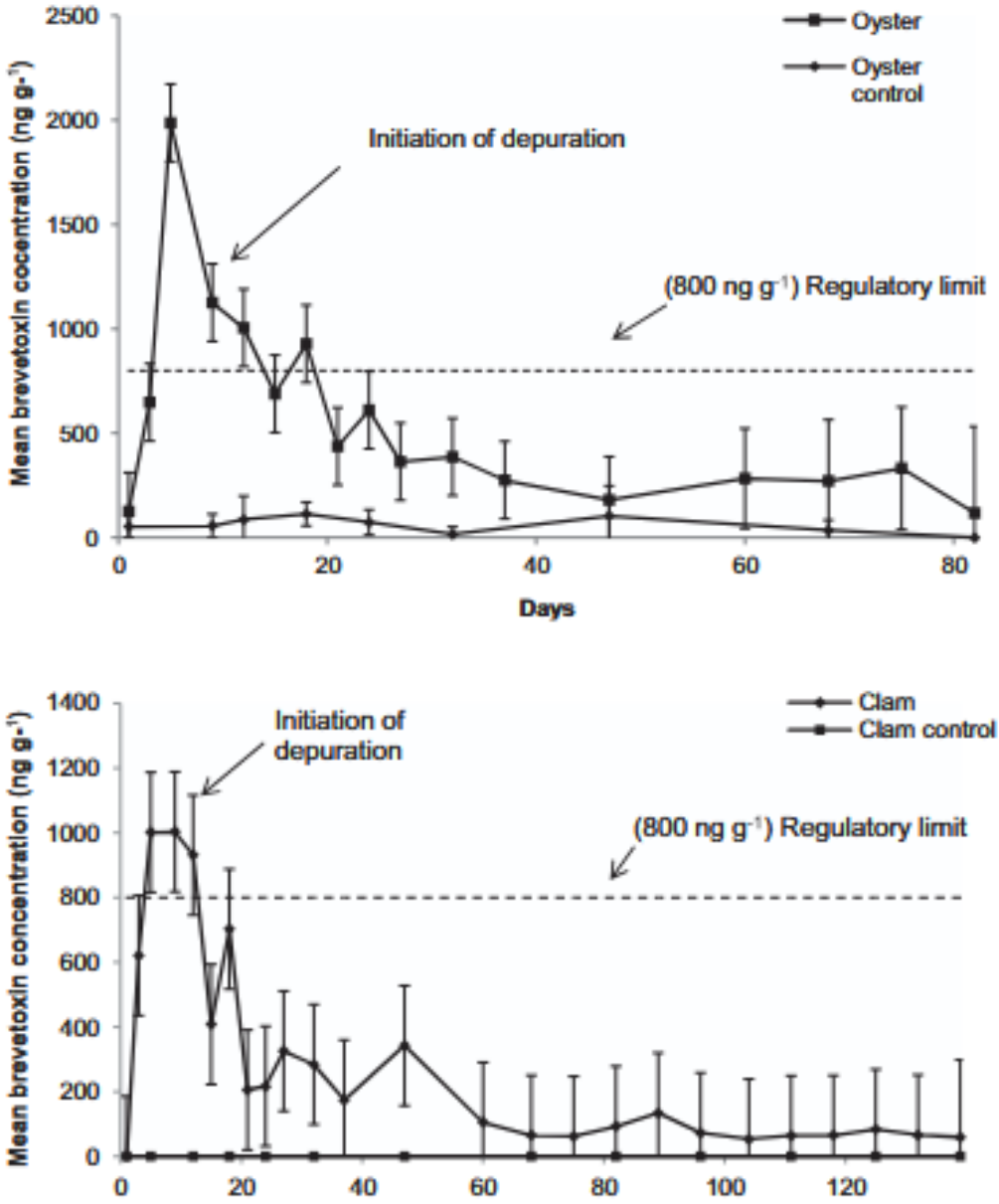



Figure 9 Uptake and elimination of BTX in Eastern oysters (*C. virginica*) and Hard clams (*M. mercenaria*). Reproduced from Griffith *et al*. (2013).

# Case Studies – *Karenia* blooms and BTX detection

## Florida *K. brevis* blooms

In Florida (USA), *K. brevis* blooms were once considered to be sporadic and seasonal, but blooms of *K. brevis* (red tides) have occurred in Florida almost annually in the years since the 1940s (Watkins *et al.* 2008). A summary of 'medium or greater' red tide events (those with *K. brevis* cell concentrations greater than 100,000 cells/L) since 1970 in Florida are shown in Table 7. Anderson *et al.* (2021) stated that whilst it appears that there might be a slight increase in the number of *K. brevis* bloom events over time, monitoring efforts have also increased. *K. brevis* blooms now regularly occur in the Gulf of Mexico, and although they are more frequent in late summer and early autumn, blooms have been documented in almost every month of the year. *K. brevis* blooms can be small, present in low concentrations (< 1,000 cell/L) and persist for only a few days or weeks, but they can also extend over large areas (6,000 km$^2$) and persist for more than 18 months (Patel *et al.* 2020).

Table 7 Historical summary of red tide events in Florida. Reproduced from Florida Fish and Wildlife Conservation Commission (https://myfwc.com/research/redtide/monitoring/database/).

Red tides with *K. brevis* >100,000 cells/L
Suspected continuance of red tide event, but not confirmed by water samples

| Year | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT | NOV | DEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1970 | | | | | | | | | | | | |
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| 2020 | | | | | | | | | | | | |
| 2021 | | | | | | | | | | | | |
| 2022 | | | | | | | | | | | | |
| 2023 | | | | | | | | | | | | |

The occurrence and magnitude of these *K. brevis* blooms are unpredictable (Brand *et al.* 2012). Some of the longest duration *K. brevis* bloom are noted below:

- In 2005, a *K. brevis* bloom persisted for over 20 months off West Florida and appeared to be fuelled by exogenous nutrient input and subsequently sustained by recycled nutrients (Heisler *et al.* 2008).
- In 2020-21 a *K. brevis* bloom along the Gulf coast of Florida lasted more than 12 months. Nutrient enrichment due to the release or phosphate and ammonium from a nearby mining site may have contributed to the prolonged bloom (Ahn *et al.* 2023).
- The longest duration *K. brevis* bloom occurred for 30 months from 1994 to 1997 (Heil and Muni-Morgan 2021).

Harmful Algae Bloom Monitoring Database - https://myfwc.com/research/redtide/monitoring/database/

The Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWRI) harmful algal bloom (HAB) database contains information on *K. brevis* blooms since 1953. The database currently consists of more than 200,000 records from samples provided by more than 190 state and county agencies, private research institutions, universities, and Florida Fish and Wildlife Conservation Commission staff. The available data include location coordinates, cell counts of *Karenia brevis* and other HAB species, and water quality measurements such as temperature, salinity and dissolved oxygen. Requests can be made to access the data.

## ***K. mikimotoi* blooms since 1930s**

*K. mikimotoi* blooms have caused mass mortalities of a range of fish, shellfish, and other invertebrates from coastal waters in several countries, including Japan, Norway, Ireland, New Zealand and Australia (Li *et al.* 2019). In a review by Li *et al.* (2019), the authors documented over 85 *K. mikimotoi* bloom events across the globe. The worldwide distribution of these *K. mikimotoi* blooms are indicated by Figure 10 and Table 8. *Karenia mikimotoi* blooms have also occurred in Coffin Bay, South Australia in 1995 and 2014 (Roberts *et al.* 2014). Locations where *K. mikimotoi* cells have been reported in the IOC.UNESCO database are also shown in Figure 11.

One unusual *K. mikimotoi* bloom occurred in an Alaskan Bay between September 2013 and April 2014 during a period in which water temperatures were as low as 2-4°C (Vandersea *et al.* 2020). The environmental and bloom conditions associated with other *K. mikimotoi* blooms are reported in Table 9. These data demonstrates the wide environmental conditions that can be observed during blooms.

From a life-cycle perspective, Liu *et al.* (2020) provided morphological and molecular confirmation that *K. mikimotoi* can produce thin-walled resting cysts. Liu *et al.* (2020) also postulated that these “resting cysts provides a possible mechanism accounting for the initiation of annually recurring blooms at certain regions and global expansion of the species during the past decades”.

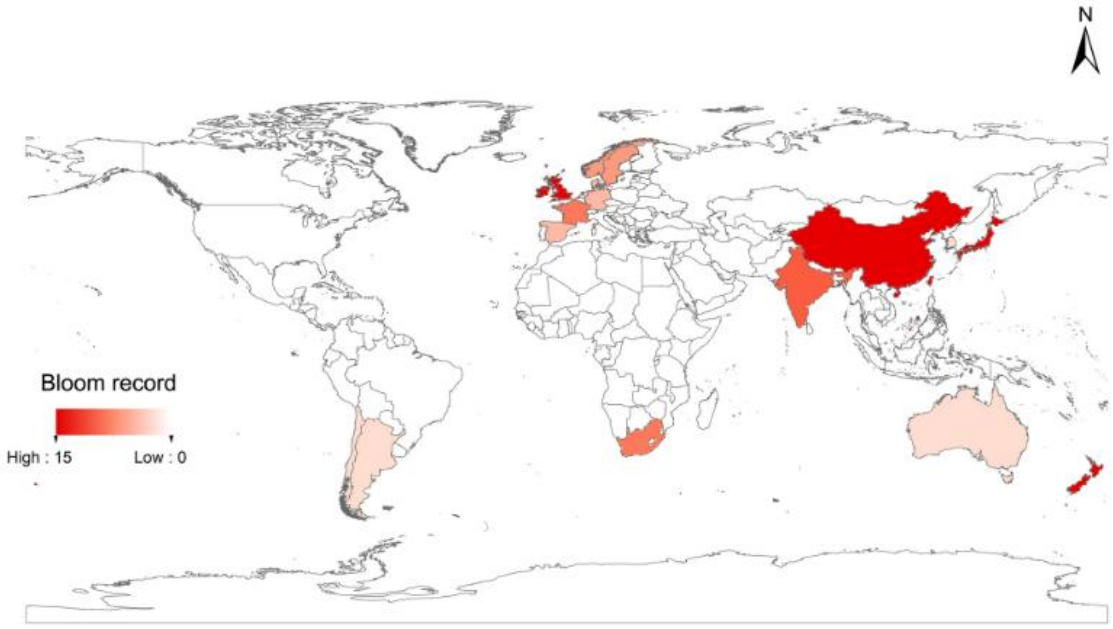


Figure 10 World distribution of blooms formed by *Karenia mikimotoi* (1934–2018). Countries coloured in red mean that bloom was recorded in their coastal waters, and degree of red mean indicates bloom frequency. Reproduced from Li *et al*. (2019).


Figure 11 Global occurrence of *Karenia mikimotoi* (brown squares). Data extracted from IOC-UNESCO Harmful Algae Information System (accessed 25 August 2025 from https://data.hais.ioc-unesco.org/).

Table 8 List of mass occurrence *of Karenia mikimotoi* with reports on adverse effects on marine life. Adapted from Li *et al*. (2019).

| Year | Area | Max cell conc. | Reports on adverse effects |
|---|---|---|---|
| **Asia** | | | |
| 1934 | Japan; Gokasho Bay, Honshu | Transparency < 0.5 m | Fish and shellfish mortality; fish gills disorder and mucus spawn |
| 1965 | Japan; Omura Bay, Nagasaki | | Fish and shellfish mortality |
| 1972 | Japan; Omura Bay, Nagasaki | | |
| 1981 | Korea; Geoje coast | $6.25 \times 10^4$ cells/mL | |
| 1985 | Japan; Suo-Nada and Iyo-Nada | Over $10^4$ cells/mL | Fisheries damage over US$ 1 million |
| 1989 | India; Kodi, Karnataka | $4 \times 10^5$ cells/mL | Fish mortality |
| 1991 | Japan; Tanabe Bay | | |
| 1992 | Japan; Hoketsu Bay | $3.9 \times 10^4$ cells/mL | |
| 1992 | Japan; Suo-Nada inshore waters | Over $10^3$ cells/mL | |
| 1993 | Japan; Suo-Nada inshore waters | $2.5 \times 10^3$ cells/mL | |
| 1994 | Japan; near Ie-shima Islands | $2.0 \times 10^3$ cells/mL | |
| 1995 | Japan; Hiroshima Bay | $1.87 \times 10^3$ cells/mL | Shellfish sustained valves and killed |
| 1998 | China; Zhujiang River Estuary and Daya Bay | | Fish kills (*Seriola* sp., *Pagrosomus major* etc.) |
| 2002 | China; Fujian coast | | Shellfish and fish kills |
| 2003 | China; coast of East China Sea | | |
| 2004 | China; Bohai Sea and East China Sea | | |
| 2004 | India; Kerala coast | 90 cells/mL | Mass mortality of fish |
| 2005 | China; coast of East China Sea and Zhujiang River Estuary | $8 \times 10^3$ cells/mL | Massive shellfish and fish kills |
| 2006 | China; coast of East China Sea | | |
| 2007 | China; Bohai Sea and East China Sea | | |
| 2008 | China; coast of East China Sea | | |
| 2008 | Japan; Suo-Nada and Beppu Bay | $4.7 \times 10^4$ cells/mL | Fish mortality |
| 2009 | China; coast of East China Sea | | |
| 2009 | India; Cochin barmouth | 56.8 mg chl a/$m^3$ | Massive fish mortality |
| 2010 | China; coast of East China Sea | | |
| 2010 | Japan; Beppu Bay | Over 16 mg chl a/$m^3$ | |
| 2011 | Singapore; Johor Straits | Over 200 cells/mL | |
| 2012 | China; coast of East China Sea | $3.4 \times 10^4$ cells/mL | Massive abalone kills and mortality of fish |
| 2013 | India; Gulf of Mannar | | Mass mortality of fish |
| 2014 | Singapore; Johor Straits | Over $5 \times 10^3$ cells/mL | |
| 2014 | Japan; Imari Bay | Over $1 \times 10^4$ cells/mL | |
| 2014 | China; coast of East China Sea | | |
| 2015 | Japan; Hakodate Bay | $1.15 \times 10^4$ cells/mL | Mortality of fish (chum salmon), squid and abalone |
| 2015 | China; coast of East China Sea | | |
| 2015 | Japan; Sasebo Bay | 800 cells/mL | |
| 2015 | India; Kochi estuary | 600 cells/mL | |
| 2018 | China; coast of East China Sea | | Mortality of fish and abalone |
| **Europe** | | | |
| 1966 | Norwegian; Oslo-Bergen | $7.0 \times 10^4$ cells/mL | Fish mortality (sea trout, rainbow trout, cod, eel, coalfish) in submerged containers |
| 1968 | Danish west coast | $8.6 \times 10^3$ cells/mL | Fish killed (several species, mainly cod). Mortality of lugworms and other invertebrates |
| 1968 | German Bight near Helgoland | $7.8 \times 10^3$ cells/mL | |
| 1971 | Irish Sea; North Wales coast, River Conwy | $5.2 \times 10^3$ cells/mL | Mortality of lugworms, *Echinus* |

| Year | Area | Max cell conc. | Reports on adverse effects |
|---|---|---|---|
| 1975 | English Channel; Southwestern part | $1.7 \times 10^3$ cells/mL | |
| 1976 | English Channel; Southwestern part | 128 mg chl a/$m^3$ | |
| 1976 | Norwegian, Oslo-Bergen | $2.3 \times 10^4$ cells/mL | Fish mortality (salmon, rainbow trout, coalfish, sprat, cod, eel, black goby); Mortality of invertebrates (lugworms, starfishes, mussels) |
| 1976 | Ireland, south coast to Youghal | | Mortality of fish and invertebrates (lugworms, ragworms, sole, plaice, flounder, eels, gapers, razor fish and cockles) |
| 1978 | England; Mounts Bay to St Austell Bay | | Mortality of fish and invertebrates; clearance rates reduction of *Mytilus edulis* |
| 1978 | Southwest Ireland; Dunmanus Bay, Roaringwater Bay | | Fish kills; Mortalities of intertidal organisms |
| 1979 | Southwest Ireland; | | Widespread mortalities of shellfish (gastropods and echinoids), fish (rainbow trout) and crabs |
| 1980 | Scotland; Sea lochs in the north of the Firth of Clyde | $2 \times 10^4$ cells/mL | Salmon killed for pumped water |
| 1981 | Swedish west coast, Gullmar fjord | $1.14 \times 10^4$ cells/mL | Fish kills |
| 1981 | Norway | $7 \times 10^4$ cells/mL | |
| 1982 | Swedish west coast | 830 cells/mL 9.5 mg chl a/$m^3$ | Fish kills |
| 1984 | Southwest Ireland; Roaringwater Bay | 500 cells/mL | |
| 1986 | England; Plymouth Breakwater | Over $1.5 \times 10^3$ cells/mL | Mortality and avoidance of young fish |
| 1987 | Southwest Ireland; Bantry Bay | ~500 cells/mL | |
| 1987 | Western English Channel | $2.56 \times 10^3$ cells/mL | |
| 1988 | Swedish west coast | 683 mg chl a/$m^3$ | |
| 1989 | Dutch; North-Sea Waters | | |
| 1989 | Spain; estuary of Pontevedra | $7 \times 10^4$ cells/mL | |
| 1990 | Denmark; Kattegat | $3.25 \times 10^3$ cells/mL | |
| 1991 | Southwest Ireland; Bantry Bay | $1.92 \times 10^3$ cells/mL | |
| 1993 | Western English Channel off Plymouth | Over $2 \times 10^3$ cells/mL | |
| 1995 | Ireland; between Sherkin Island and Cork Harbour | $4 \times 10^3$ cells/mL | |
| 1998 | Ireland; Galway Bay | Over 900 cells/mL | |
| 1999 | Ireland; west and southwest of the Aran Islands | 750 cells/mL | |
| 2001 | Spain; Ria de Vigo | $3 \times 10^3$ cells/mL | |
| 2003 | Western English Channel | Over $1 \times 10^3$ cells/mL | |
| 2004 | Western English Channel; Southwest Approaches | | |
| 2005 | Northern half of the western Irish coastline | | Major mortalities of benthic and pelagic marine organisms; fish and shellfish kills |
| 2006 | Scottish waters | Over $1 \times 10^3$ cells/mL | Extensive mortalities of benthic organisms including annelids and molluscs and some species of fish. Farmed fish mortalities were absent, but gill damage was reported. |
| 2009 | England; coasts of Cornwalland Devon | $5.4 \times 10^3$ cells/mL | Dead marine animals (dogfish, turbot, eel, dover sole and sea potatoes) |
| 2010 | English Channel; Western approaches | 14 mg chl a/$m^3$ | |
| 2011 | UK; Brings Deep, Orkney Islands | Near $1 \times 10^3$ cells/mL | |
| **Oceania** | | | |

| Year | Area | Max cell conc. | Reports on adverse effects |
|---|---|---|---|
| 1981 | New Zealand; Tasman Bay | | |
| 1986 | New Zealand; Tennyson Inlet | | |
| 1987 | New Zealand; Tennyson Inlet | | Salmon kill |
| 1989 | Australia; south-east Tasmania | | Fish kills |
| 1993 | New Zealand; northeast coast of North Island | | |
| 1994 | New Zealand; Southland area | | Toxic shellfish event |
| 2002 | New Zealand; Orewa, Whangaparaoa, and eastern Coromandel Peninsula | | Mortalities of fish (parore, flounder, yellow-eyed mullet, eel, goby and spotty) and abalone |
| 2003 | New Zealand; Tasmanian | | Mortality of Atlantic salmon |
| **Africa and America** | | | |
| 1982 | Argentina; estuary of Río de la Plata | | |
| 1988[9] | South Africa; False bay | $1.7 \times 10^4$ cells/mL | Faunal mortalities (sardine, alikreukel, octopus, chitons and starfish) and eye, nose, and lung irritations in bathers and fishermen |
| 1989[9] | South Africa; False bay | $2.4 \times 10^4$ cells/mL | Faunal mortalities and eye, nose, and lung irritations in bathers and fishermen, great losses in abalone culture |
| 1996[9] | South Africa; Walker bay | | Mortality of abalone larva |
| 1999 | Chile; Southern coast | $8\text{-}9 \times 10^3$ cells/mL | High mortality in shellfish (clam, sea urchins, abalone), farmed salmon and wild fish species |
| 2016 | South Africa; | 516 cells/mL | |

[9] Bloom also contained *K. cristata* (Botes *et al.* 2003).

Table 9 Environmental and bloom conditions of globally occurring *Karenia mikimotoi* blooms. Reproduced from Vandersea *et al*. (2020).

| Location | Cell concentrations and prevailing conditions during bloom | | | Range over which cells were observed | | Conditions favouring bloom Formation |
|---|---|---|---|---|---|---|
| | ***K. mikimotoi* (cells/L)** | **Temperature (˚C)** | **Salinity** | **Temperature (˚C)** | **Salinity** | |
| Kachemak Bay, Alaska, USA | 1,000 - 19,900,000 | 8 - 12 | 24.8 – 31.8 | 12 - 14 | 21 - 32 | Stratification and nutrient rich waters accessible below thermocline |
| Imari Bay, Japan | 10,000 - 10,000,000 | 26 - 29 | 32 - 34 | | | Stratification due to freshwater inflow coupled with upwelling induced delivery of high nutrient bottom water and by limited water exchange |
| English Channel | 200,000 - 1,500,000 | 12 - 17 | 33.9 - 35.5 | | | Stratification due to high runoff delivering increased nutrients |
| Gulf of Lawrence, Canada | 2,000 - 990,000 | 12 -13 | 17 - 29 | 4 -19 | 15 - 31 | Strong vertical stratification, shallow thermocline, presence of an estuarine front, and availability high ammonia concentrations |
| Carmans Estuary, Long Island Sound, New York, USA | >1,000 - 10,200,000 | 24.5 - 29 | 6-20 | 19 - 30 | 0 - 22 | Strong vertical stratification |
| Norwegian waters | 500,000 - 10,000,000 | 21 | 22.3 | 5-22 | Dec-35 | Blooms in stratified waters along frontal zones |
| Coast of Thiruvananthapuram, west India | 2,000 - 94,000 | 28.2 - 28.5 | 33.9 - 35.7 | | | Stratification due to heavy runoff delivering increased nutrients |
| English Channel | 700,000 - 1,380,000 | 16 - 18.5 | 35.05 - 35.2 | | | Stratification due lower salinity intrusions and calm winds |
| English Channel | 69,000 - 2,649,000 | 12.5 - 16 | 34.6 - 35.1 | | | Intense stratification |
| Gokasho Bay, Japan | 1,000 - 4,500,000 | 12 -27 | | | | - |
| Ria of Pontevedra, northwest Spain | 8,908,000 - 69,337,000 | 19 - 19.5 | 35.02 - 35.10 | | | Stratification and nutrient rich waters accessible below thermocline |
| Western English Channel | 39,810 - 1,585,000 | 14 - 16 | | 12-16 | | Stratification |
| East Johor Straits between Peninsular Malaysia to the north and Singapore to the south | 1,350,000 - 8,370,000 | 27.4 ± 1.41 | 21 - 25 | | | Runoff decreased salinity, and input of high levels N and P |
| Changjiang River estuary and adjacent waters, China | 64,000 - 8,730,000 | 17.2 - 19.3 | 24.3 - 30.6 | | | Correlated with reduced salinity, increased nutrient fluxes, shallow depth, and sheltered conditions |
| Sundays Estuary, South Africa | 516,000 | 22 - 23.5 | Oct-20 | | | Stratified water column concomitant with nutrient-enriched mesohaline (~ 10 m) surface waters |

| | | | | | | |
|---|---|---|---|---|---|---|
| Swedish coast | 10,000 - 810,000 | 14 - 16 | 24 - 30 | | | Strongly stratified water column; bloom develops along pycnocline where sufficient nutrients available then overtime migrates up in water column eventually forming dense surface blooms |
| Cochin inlet, Southwest coast of India | 400 - 15,500,000 | 30.5 - 31.2 | 22.5 - 29.8 | | | Thermally stratified |
| Seto Inland Sea, Japan | 2,000 - 380,000 | 22 - 24 | 30.3 - 33 | | | Stratified, shallow thermocline (10-15 m) with accessible nutrient rich waters below thermocline |
| West and northwest coasts of Ireland, | 5,000 - 3,700,000 | 13.0 - 17.1 | 34.2 - 34.9 | | | - |
| Términos Lagoon, Campeche, Mexico, SE Gulf of Mexico | 1000 - 9,000 | 27.8 - 28.4 | 17.4 - 19.5 | 27.8 - 30.6 | 11.6 - 19.5 | Shallow water column (ave. 3.5 m), no vertical stratification, but low winds and abundant nutrients |
| English Channel | 5,000 - 8,000,000 | 19 - 22 | 34.8 - 35.0 | 12 - 22.5 | 34.6 - 35.8 | Stratification due to unusually high summertime water temperatures >19C, favourable winds, and intrusion of low salinity water from French rivers into the English Channel |
| Southwestern Ireland | 1,000 - 530,000 | 16 | 35 | 14.5 - 16 | 35.1 - 35.4 | Strong thermal stratification and favourable wind stress - brought nutrient replete cells from sub-surface maxima along thermocline of waters in the northern Celtic Sea nearer surface where conditions more favourable or growth. |
| Southeast Arabian Sea, bordering western India | 189,400 -193,700 | 28.2 - 28.5 | 33.9 - 35.7 | | | Formed in thermally stratified cyclonic eddies when windspeeds (turbulence) were low but still sufficient to cause onshore accumulation in an embayment where runoff from heavy rainfall supplied nutrients |
| Southeastern Arabian Sea, India | 600,000 | 24.2 - 27.0 | 33.6 - 34.8 | | | Formed just outside inlets in stratified water formed from outflow of N-rich water from the inlets during summer monsoon |
| Hiroshima Bay, Japan | 1,000 - >10,000,00 | 22.5 - 24.8 | 27.2 - 30.5 | 22.2 - 27.0 | 21.3 - 31.0 | Highly stratified |
| Changjiang (Yangtze River) Estuary, China | 20,000 - 909,900 | 18 - 22 | 27 - 32 | | | Stratified stations, characterized by low nitrate, low phosphate and turbidity - dense accumulation of cells along the isohaline with access to nutrients below |

## New Zealand 1992-93 and 1998

Various investigations have concluded that it is not known for certain what the circumstances were that led to the development of the New Zealand *Karenia* spp. bloom and subsequent NSP event in 1992-93. It has been postulated that the bloom species were "members of a hidden plankton flora (previously present in low concentrations), which developed into bloom proportions triggered by unusual climatic conditions (higher than usual rainfall, lower than usual temperature) coincident with an El Niño event" (Hallegraff 2004). During the 1992-93 outbreak NSP toxin levels reached 592 MU/100g for edible shellfish (Australia New Zealand Food Authority 2001). The apparent identification of brevetoxins in shellfish samples was through the mouse bioassay and presence of *Karenia* spp. that were in some cases initially misidentified as *K. brevis* (Todd, 2022). In 2000, Hay *et al.* (2000) concluded that the identity of the causative agent in the 1992-93 NSP event is uncertain: both *Gymnodinium c.f. breve* (hereafter *Gymnodinium papilonacea*) and *Gymnodinium c.f. mikimotoi* (hereafter *Gymnodinium selliformis*) were at the time present in elevated numbers. Later McNabb *et al.* (2006) also stated that the source of the toxin was still speculative. A review of the New Zealand outbreak implicated *K. mikimotoi* as the likely causative agent, but other suspect species were also present in the bloom (Watkins *et al.* 2008). *Karenia mikimotoi* was the dominant *Karenia* spp. in the bloom, though the source of the brevetoxin has not yet been identified (Rhodes and Smith 2022).

The rates of toxin elimination from cultivated Greenshell mussels following the 1992-93 bloom were slow, and a residue of the toxicity existed two months after the disappearance of the dinoflagellates from the plankton (MacKenzie 2008).

In 1993, following the bloom, New Zealand implemented a comprehensive marine biotoxin monitoring program. Traces of BTXs are reported to appear seasonally within shellfish from the Hauraki Gulf region (New Zealand) but there have been no clear association with causative phytoplankton, and BTX concentrations in these shellfish have never exceeded regulatory action levels (MacKenzie 2008). However, it has also been reported that between September 1994 and July 1996, 0.2 percent of shellfish samples collected from around the coastline of New Zealand showed a NSP toxin level above the regulatory limit (Australia New Zealand Food Authority 2001).

In 1998 a toxic algae bloom also impacted a large area of Wellington Harbour. A summary of this event is reported below. Later investigations subsequently isolated *K. brevisulcata* (Chang) from this bloom (Holland *et al.* 2012).

**1998 Wellington Harbour Event – Reproduced from FAO (2004)**

"Immediately after a series of fish and marine fauna kill episodes and outbreaks of human respiratory illness being reported off Wairarapa coast and Hawke Bay on the North Island east coast, Wellington Harbour experienced a severe toxic outbreak that persisted from mid-February to April 1998. The outbreak decimated almost all marine life (including seaweeds) in the harbour. During this unusual outbreak, eels and flounders were first noticed as the major harbour kills, which then spread across to kills of other pelagic fish and marine invertebrates. Eighty-seven people in Wellington Harbour reported suffering from respiratory illness; beach goers, swimmers, and wind-surfers all complained of a dry cough, a severe sore throat, running nose and skin and eye irritations. Furthermore, hatchery workers and divers complained among other symptoms also of severe headaches and a facial sunburnt sensation. The unprecedented bloom was found to be dominated by a non-described *Gymnodinium* sp. (33.3 x $10^6$ cells/litre). The morphological characters of this new species look like the Japanese *Gymnodinium mikimotoi*. The Wellington Harbour toxin was stable in both alkaline and acidic conditions, but was not stable in weak acid. This makes it less likely to pose any human healthy risk when it is eaten. When heated to 100°C the toxin lost most of its toxicity. The toxin is also highly oxidisable and therefore can be destroyed by ozonation. One of the notable features of the 1998 Wellington Harbour bloom was the build-up of extensive sea-foam, persisting for several weeks. The impacts of this new *Gymnodinium* sp. on marine life certainly are more severe than those caused by *G. mikimotoi* from Japan, *G. breve* from the Atlantic coast of the United States, *G. cf. mikimotoi* from Western Europe and *G. galatheanum* from the North Sea. In terms of impacts of airborne and waterborne toxins on humans, this new *Gymnodinium* sp. is quite like those of *G. breve* from the Atlantic coast of the United States and *Gymnodinium* sp. recently reported for South Africa."

## France – detection of BTXs in 2018

In 2018, BTXs were detected for the first time in Europe as part of the EMERGTOX network – a French monitoring program for the detection of emerging marine biotoxins in local shellfish (mussels, oysters and clams). In November 2018, a BTX concentration of 0.117 mg/kg was detected within the digestive gland of Blue Mussels from the Corsica region (France) (Arnich *et al.* 2021).

During the monitoring program a maximum level of 0.057 mg/kg (sum BTX-2 and BTX-3) was reported in the flesh of Blue Mussels in November 2020. Associated water samples collected for phytoplankton monitoring did not detect the presence of *K. brevis* but revealed the presence of other harmful algae including *K. mikimotoi, K. papilionacea, K. longicanalis, Karenia sp, Fibrocapsa japonica and Heterosigma akashiwo*. These other species of dinoflagellates are suspected, according to the literature, of being potential producers of brevetoxin-type bioactive compounds have been present recurrently on all of the French metropolitan coasts for several years (ANSES 2021).

In 2020 ANSES, the French Agency for Food, Environmental and Occupational Health & Safety, received a request from the French Directorate General for Food and the French Directorate General for Health to provide their expert opinion in answering five brevetoxin management or human health related questions. ANSES established an expert working group and the five questions and their responses may be relevant to the situation surrounding the South Australian bloom. In completing this request, the ANSES expert working group undertook a systematic literature review for “brevetoxin” in Scopus and Pubmed databases and identified over 850 references. This highlights the vast quantum of information that is available on the issue.

**Question 1. What toxicological data are available for brevetoxins? Do these data enable ANSES to propose health-based guidance values for acute and chronic oral exposure?**

The data in the literature from oral acute toxicity studies in animals are very limited and do not enable a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL) to be identified.

The working group identified two lowest levels associated with systems “acute LOAELs”; 27-40.5 MU/person based on McFarren et al. (1965) and 0.3-0.4 MU/kg body weight reported by Hemmert (1975).

Data was too limited to be able to establish an acute reference dose (ARfD).

To the knowledge of the working group, no repeated oral administration toxicity studies are available, so it is not possible to propose a chronic toxicological reference value.

**Question 2. Based on the toxicological data identified by ANSES, is it possible to propose a guidance level in shellfish above which further investigations would be required as part of the EMERGTOX network?**

The working group considered that the maximum level of 0.8 mg BTX-2/kg shellfish flesh does not appear protective enough and recommends a guidance level of 0.18 mg BTX-3 equivalent/kg shellfish flesh (corresponding to the sum of all tested BTX metabolites).

The working group selected BTX-3 (and not BTX-2) as the reference BTX in shellfish. BTX-3 is the reference BTX for the ELISA test; it has a lower $LD_{50}$ than BTX-2. Moreover, BTX-3 occurs in larger quantities in shellfish compared to BTX-2. The limit of 20 MU/100 g or 0.8 mg BTX-2/kg corresponds to 0.68 mg BTX-3/kg shellfish flesh.

**Question 3. What methods could be recommended for monitoring brevetoxins in marine environments as part of the EMERGTOX network? What further investigations would be required if the guidance level was exceeded?**

Working group believes current monitoring of French coastal waters is appropriate, i.e. once a fortnight in routine conditions, and once a week if the alert threshold is exceeded for microalgae producing

regulated marine biotoxins. The working group also recommended weekly samples if BTX concentrations are quantified in shellfish at a given location.

Monthly sampling of shellfish also occurs and if the BTX guidance level of 0.18 mg BTX-3 equivalent/kg shellfish flesh is exceeded the working group recommended increasing monitoring for BTXs in shellfish and actively providing information to health professionals to assist in the detection of any potential cases of NSP.

**Question 4. Given the toxicological data and in view of the context, is there a public health concern associated with shellfish consumption based on the levels of contamination identified in certain marine areas of France?**

The maximum concentration reported to date is 0.345 mg/kg digestive gland for the sum of BTX-2 and BTX-3 (November 2020), which corresponds to an estimated value of 0.057 mg/kg total flesh for the sum of BTX-2 and BTX-3 (assuming the BTXs were solely present in the digestive gland/based on preliminary results).

The working group notes that the concentrations of the two BTXs in shellfish increased from 0.02 mg/kg total flesh in November 2018 to 0.044 mg/kg in January 2019 and then to 0.057 mg/kg in November 2020.

Recommended expanding analysis to include BTX-1, BTX-2, BTX-3, BTX-B1, BTX-B2, S-desoxy-BTX-B2, BTX-B3, and BTX-B4(a and b).

Could not rule out acute exposure, or potential effects associated with repeated exposure to low concentrations.

**Question 5. Is there a health concern related to exposure to brevetoxins via direct contact with contaminated water, associated in particular with swimming, water activities or inhalation of sea spray?**

The BTX concentration in aerosols collected on the beach depends on the BTX concentration in the surf zone, wind speed and direction, and beach exposure. The concentration of analogues in seawater varies depending on the phase of the bloom cycle (growth, steady state, or decline), bloom intensity, the site where it develops, and environmental conditions. BTX-2 and BTX-3 appear to be present at higher concentrations in the scum (1.9 mg/L and 1.2 mg/L, respectively ) than in the water column (breaking zone – 0.02 mg/L and 0.004 mg/L, respectively).

Several studies describe the symptoms observed during exposure to sea spray containing of BTX. Exposure to atmospheric concentrations of around 3 to 5 ng/m³ are sufficient to observe upper respiratory tract symptoms. BTX concentrations of 30 to 40 ng/m³ are necessary to cause symptoms related to lower respiratory tract involvement, particularly in asthmatics.

# Potential bloom mitigation strategies

Strategies to manage HABs generally consist of either prevention, control or mitigation. A range of physical, chemical and biological approaches have been trialled across the world to help control or manage algal blooms once they have formed. Some of these methods can be costly, pollute the environment, have regulatory requirements, social acceptance, or be challenging to utilise in natural environments, especially in large-scale blooms (Anderson *et al.* 2001). Consideration is also required when attempting to control or manage a bloom, as the disruption of algal cells can release toxic compounds into the water, or result in localised oxygen-depletion (hypoxia) conditions. No single approach is applicable for all situations, and an assessment needs to be undertaken including a no-treatment option. Mitigation techniques include but are not limited to:

- Recruiting competitors that slow the bloom's development and growth; such as seaweeds, microalgae, filter-feeders, parasites and viruses.
- Treatment with chemical algicides; such as peroxides, ozone, copper.
- Deep-water upwelling, skimming or flocculation.
- Clay/Modified Clay -

The following provide a brief summary from prominent studies that have focused on the control of *K. brevis* or *K. mikimotoi* blooms.

## Clay/Modified Clay

Clays are fine-grained, natural earth minerals and are surface active substances that can interact with microalgae forming flocculants that can sink. Clay flocculation can act throughout the water column and have been widely used in several countries including China and South Korea. Clay flocculation trials have occurred in USA but have not been adopted due to a current lack of data on environmental impact. Some clays have been modified through the additional of polymers, oxidants or other materials to improve efficacy in removal of various HAB species. In 2014, modified clay treatment was included as a Chinese national standard method for the treatment of red tides (GB/T 30743-2014) (Zhang *et al.* 2022). Some modified clays have also been reported to have a good ability at removing *K. mikimotoi or K. brevis* cells, as well as brevetoxins (Pierce *et al.* 2004, Liu *et al.* 2018, Zhang *et al.* 2022).

## Ozone

Ozone is a highly reactive gas and a strong oxidising agent. Ozonated seawater can inactivate toxins associated with dinoflagellate blooms, as well as in reducing the toxin levels accumulated in shellfish (Schneider *et al.* 2003). When ozone contacts seawater, it reacts with indigenous bromide to form hypobromite and hypobromous acid. These by-products act as secondary oxidants and can broaden ozone's disinfection efficacy. Further ozonation can result in the formation of bromate (a persistent disinfection byproduct and suspected human carcinogen regulated in many jurisdictions), and in the presence of natural organic matter, bromoform and bromamines can also be formed. Bromoform and bromamines can have some disinfectant properties, but are also irritants at high levels.

The use of ozone to detoxify shellfish or minimally, its utilization to treat incoming seawater to a depuration or wet storage facility, could reduce the risk of cross contamination in areas where a bloom might have occurred. However, ozone can be toxic to humans and aquatic organisms and concentrations must be carefully managed.

## Peroxides

Peroxide-based algaecide treatments were considered promising methods to control HABs (Hu *et al.* 2022). Hu *et al.* (2022) demonstrated that several hydrogen peroxide-based algaecides (registered by the US Environmental Protection Agency) could be effective against *K. brevis* cells and exhibited some reduction in brevetoxin levels.

### Algicidal bacteria

Interactions between microalgae and bacteria are complex and can range from mutually synesthetic to antagonistic. Antagonistic bacteria interactions can be algicidal (inhibit growth and/or kill algae) or algistatic (inhibit algae growth). A review on algicidal bacteria for the control of HABs was recently published by Coyne *et al.* (2022). Prior to the widespread use of algicidal bacteria to control HABs, the biosafety and biosecurity risks need to be evaluated. Several examples of the use of algicidal bacteria have been published. For example, Shi *et al.* (2022) reported that an algicidal powder containing a particular *Pseudoalteromonas* strain (previously isolated from a *K. mikimotoi* bloom event) demonstrated high activity against indoor algal culture and microcosm of nature algal blooms dominated by *K. mikimotoi* and *Karlodinium* spp.. Immobilising a *Shewanella* strain on different matrices and testing the effectiveness against several harmful dinoflagellates with a goal to use the technology against *K. brevis* blooms has also been investigated (Wang *et al.* 2025).

### Biochar-based coagulants

Chambers and Reza (2024) investigated biochar-based coagulants and demonstrated the versatility in mitigating *K. brevis* blooms through flocculation and brevetoxin removal. Some scientific findings were presented by Lovko *et al.* (2024) at the 12th US Symposium on Harmful Algae (Oct 27-Nov 1, 2024 in Portland, Maine).

# Brevetoxins in non-bivalve seafood

ANSES (2021) reported that several studies have demonstrated the contamination of fish by brevetoxins. However, in Florida there is no routine monitoring for brevetoxins in fish exposed to *K. brevis* blooms and there have been no cases of human poisoning linked to the consumption of BTX-contaminated fish reported to date (Abbott *et al.* 2021). Whilst BTX levels typically do not accumulate in fish muscle at concentrations to cause a human illness, fish can concentrate BTXs in their viscera (Naar *et al.* 2007, Abbott *et al.* 2021). A study by Abbott *et al.* (2021) reported that if fish are well cleaned and if only the fillet is eaten, then consumers should not become sick, whilst Naar *et al.* (2007) reported that acute intoxication could occur from consuming contaminated fish viscera, such as from whole fish.

An environmental assessment undertaken in Florida (USA) after two NSP cases were reported following the consumption of recreationally harvested Horse Conchs, collected one Horse Conch, one Lightning Whelk, two Banded Tulips, and a Sunray Venus Clam from the approximate location of the original site. The shellfish samples were submitted to a US FDA laboratory for testing where brevetoxins were detected in the muscle and viscera of these individual samples. A summary of the BTX concentrations from this investigation is reported in Table 10. Higher levels were detected in the viscera of the Horse Conch, Lightning Whelk and Banded Tulips (and above the guidance level of 0.8 mg/kg) as compared to the muscle tissue. The Sunray Venus Clam also had brevetoxins significantly above the 0.8 mg/kg level.

Table 10 Detection of brevetoxins in Horse Conch, Lightning Whelk, Banded Tulips and Sunray Venus Clams. Adapted from Florida Department of Health (2018).

| Sample | Brevetoxin concentration (mg/kg) | | |
|---|---|---|---|
| | Muscle | Viscera | Whole |
| Horse Conch | 0.03 | 1.77 | - |
| Lightning Whelk | Trace | 5.23 | - |
| Banded Tulip A | 0.3 | 10.33 | - |
| Banded Tulip B | 0.21 | 58.15 | - |
| Sunray Venus Clam | - | - | 13.88 |

Naar *et al.* (2007) presented BTX levels from 184 fish representing 38 species of varying diets and trophic groups that were collected off the coast of Florida between March 2004 and November 2006. The maximum BTX concentrations in the muscle, liver and gastrointestinal (GI) content from this study are reported in Table 11. A significant *K. brevis* bloom occurred during the sampling program (*K. brevis* bloom: July-November 2005). Naar *et al.* (2007) reported that brevetoxins persisted in finfish, particularly livers, with an average brevetoxin concentration in the livers of approximately 0.1mg equivalent BTX-3/kg a year after bloom cessation. In a subsequent review, Watkins *et al.* (2008) noted that planktivorous finfish have been shown to retain brevetoxins in the muscle, gut and organs, but at much lower concentrations than in molluscs. Watkins *et al.* (2008) stated that the public health implications of this finding cannot be evaluated as additional research on the human toxicity of these lower doses is needed. It was also acknowledged that the practice of eating whole fish (in some Asian communities and other cultures) may put individuals at risk for brevetoxin-poisoning as the highest levels of brevetoxins in finfish have been found in the fish liver and stomach contents (Watkins *et al.* 2008).

Pérez Linares *et al.* (2009) demonstrated that in an experimental study Vannamei Prawn (*Litopenaeus vannamei*) are an unconventional vector of BTXs and therefore can represent a potential risk for human health. Whilst the magnitude of the risk was not reported, the authors measured BTX concentrations well below the toxicity threshold (0.8 mg equivalent BTX-2/kg) with values between 0.08 and 0.09 mg equivalent BTX-2/kg in the digestive glands and muscle, respectively. This level of contamination appeared 45 days after exposure of a “relatively low” cellular concentrations of *K. brevis* of $10^3$ to $10^6$ cells/L.

As part of FRDC Project 2014/032 (Improved understanding of Tasmanian harmful algal blooms and biotoxin events to support seafood risk management) a systematic literature review was undertaken in 2014/15 to identify seafood species known to bioaccumulate neurotoxic shellfish toxins (NSTs). Apart from bivalves, studies have reported BTXs in a range of gastropods, fish, sea slugs as well as in stranded marine animals (dolphins, manatees, sea turtles) and birds (cormorants, sanderlings, turnstones). A summary of the BTX levels from this literature review is reported in Table 12.

Table 11 Maximum BTX concentrations measured in fish collected from St Joseph Bay between March 2004 and November 2006. Reproduced from Naar *et al*. (2007).

| Species | Common name | Main diet | Number of specimens | Maximum [PbTx-3 eq. (ng $g^{-1}$)] | | |
|---|---|---|---|---|---|---|
| | | | | Muscle | Liver | GI contents |
| *Sardinella aurita* [a,b] | Spanish sardine | Plankton | 6 | 581 | <ld | 137 |
| *Mugil cephalues* [a] | Striped mullet | Plankton | 9 | 40 | 6682 | 393 |
| *Harengula jaguana* | Scaled sardine | Plankton | 13 | 52 | 2472 | 2839 |
| *Opisthonema oglinum* | Atlantic thread herring | Plankton | 21 | 54 | 473 | 508 |
| *Remora remora* | Remora | Plankton | 1 | <ld | 131 | 384 |
| *Lagodon rhomboides* [a] | Pinfish | Herbivore | 45 | 129 | 1453 | 911 |
| *Hyporhamphus meeki* [a] | American halfbeak | Invertebrates | 5 | 124 | 1977 | 188 |
| *Chilomycterus schoepfi* [a] | Striped burrfish | Benthic invert. | 1 | 27 | 571 | 138 |
| *Acanthostracion quadricornis* | Scrawled cowfish | Benthic invert. | 2 | 20 | 228 | 52 |
| *Bairdiella chrysoura* | Silver perch | Benthic invert. | 1 | <ld | 246 | 37 |
| *Orthopristis chrysoptera* | Pigfish | Benthic invert. | 3 | <ld | 277 | 105 |
| *Syngnathus scovelli* | Gulf pipefish | Benthic invert. | 1 | <ld | <ld | <ld |
| *Archosargus probatocephalus* | Sheepshead | Benthic invert. | 5 | 18 | 3709 | 613 |
| *Haemulon plumierii* | White grunt | Benthic invert. | 1 | <ld | 19 | <ld |
| *Lobotes surinamensis* | Tripletail | Benthic invert. | 1 | <ld | <ld | <ld |
| *Ariopsis felis* | Hardhead sea catfish | Benthic invert. | 2 | 12 | 1408 | 27 |
| *Bagre marinus* | Gafftopsail catfish | Benthic invert. | 1 | <ld | 1312 | <ld |
| *Leiostomus xanthurus* | Spot | Benthic invert. | 27 | 186 | Viscera 386 [c] | |
| *Menticirrhus americanus* | Southern kingfish | Benthic invert. | 3 | <ld | 22 | 8 |
| *Menticirrhus littoralis* | Gulf kingfish | Benthic invert. | 2 | <ld | Viscera 31 [c] | |
| *Menticirrhus saxatilis* | Northern kingfish | Benthic invert. | 7 | <ld | Viscera 77 [c] | |
| *Micropogonias undulatus* | Atlantic croaker | Benthic invert. | 6 | <ld | 51 | 57 |
| *Urophycis floridana* | Southern codling | Benthic invert. | 4 | 60 | Viscera 285 [c] | |

| Species | Common name | Main diet | Number of specimens | Maximum [PbTx-3 eq. (ng $g^{-1}$)] | | |
|---|---|---|---|---|---|---|
| | | | | Muscle | Liver | GI contents |
| *Prionotus tribulus* | Bighead sea robin | Benthic invert. | 1 | <ld | <ld | <ld |
| *Elops saurus* [a] | Ladyfish | Piscivore | 5 | 11 | 76 | 49 |
| *Cynoscion nebulosus* [a] | Spotted seatrout | Piscivore | 16 | 414 | 5133 | 3955 |
| *Lutjanus griseus* [a] | Mangrove snapper | Piscivore | 10 | 52 | 321 | 615 |
| *Mycteroperca microlepis* [a] | Gag grouper | Piscivore | 6 | 116 | 6034 | 720 |
| *Opsanus beta* [a] | Gulf toadfish | Piscivore | 1 | 69 | 7 | 400 |
| *Lutjanus campechanus* [a] | Red snapper | Piscivore | 3 | 102 | 16483 | 664 |
| *Paralichthys albigutta* [a] | Gulf flounder | Piscivore | 5 | 65 | 528 | 742 |
| *Carangoides bartholomaei* | Yellow jack | Piscivore | 1 | <ld | 218 | <ld |
| *Caranx crysos* | Blue runner jack | Piscivore | 1 | <ld | 478 | <ld |
| *Pomatomus saltatrix* | Bluefish | Piscivore | 18 | 45 | 190 | 131 |
| *Scomberomorus maculatus* | Spanish mackerel | Piscivore | 28 | 24 | 221 | 11 |
| *Strongylura marina* | Atlantic needlefish | Piscivore | 1 | <ld | <ld | <ld |
| *Centropristis striata* | Black sea bass | Piscivore | 10 | <ld | 52 | 206 |
| *Cynoscion arenarius* | Sand seatrout | Piscivore | 1 | <ld | 468 | 14 |
| *Lutjanus synagris* | Lane snapper | Piscivore | 1 | <ld | <ld | 16 |
| *Oligoplites saurus* | Leatherjacket | Piscivore | 4 | <ld | 109 | 33 |
| *Paralichthys lethostigma* | Southern flounder | Piscivore | 2 | <ld | 8 | <ld |
| *Synodus foetens* | Inshore lizardfish | Piscivore | 4 | <ld | 96 | 12 |

<ld, below level of detection.

[a] At least one specimen collected during September or November 2005 (i.e. during or just after *K. brevis* bloom).

[b] No liver or GI contents analysed from *K. brevis* bloom period.

[c] Fish collected only in 2004, muscle and viscera (organs and GI tract) analysed.

Table 12 Neurotoxic shellfish toxins (NST) in non-bivalve seafood of relevance to Tasmania, Australia. Literature review undertaken 2014/15 as part of FRDC Project 2014/032. Reproduced from Turnbull *et al*. (2015).

| **Species** | **Country** | **Maximum NST mg Pb-$TX_2$ /kg (maximum level in bivalves 0.8 mg/kg)** | **Study type (lab/field/method)** |
|---|---|---|---|
| **Abalone** | | | |
| *Haliotis iris* | New Zealand | Not Detected (n=331) | Risk Profile |
| Greenlip (*H. laevigata),* blacklip (*H. rubra)* and roe (*H. roei)* abalone | Australia | Not Detected, NRS survey (n=52) | Risk Profile |
| **Other Gastropods** | | | |
| Lightning whelk (*Busycon contrarium),* and Florida fighting conch (*Strombus alatus*) | USA | 0.54 whelk, 0.04 conch | Field study |
| Whelks (*Busycon contrarium)* | USA | 22 digestive tissue | Case study |
| **Fish** | | | |
| Pinfish (*Lagodon rhomboids),* pigfish (*Orthopristis chrysoptera)*, striped mullet (*Mugil cephalus)* and spot (*Leiostomus xanthurus)* | USA | 10.844 in liver of Spot fish<br>0.433 in whole pinfish<br>0.009, 0.258, 1.838, 0.113, 0.168 in Striped mullet muscle, viscera, liver, gill, & stomach respectively | Field study |
| 22 species - most common Blacktip sharks (*Carcharhinus limbatus*), atlantic sharp-nose bonnethead *(Sphyrna tiburo*), and lemon shark (*Negaprion brevirostris)* | USA | 27.7 in liver black nosed shark (*Carcharhinus acrontus*), low in muscles of all, except one lemon shark (0.755) | Field study |
| Juvenile striped mullet *(Mugil cephalus)*, 38 spp. in total | USA | Striped mullet (n=9) max 0.040, 6.682, 0.393 in muscle, liver and GI contents. Found in 65% fish sampled, found in muscle and viscera, highest in liver of red snapper (16.483) & meat of sardine (0.581) | Field and lab experiment |
| Lemon shark *(Negaprion brevirostris)* | USA | 3.112 in sardine liver | Field study |
| Salmon (*Oncorhynchus tshawytscha*), snapper *(Pagrus auratus)* | New Zealand | Not measured | Lab study |
| Striped mullet (*Mugil cephalus*) | USA | Detected in blood | Feeding study |
| Thread herring (*Opisthonema oglinum*), scaled sardine *(Harengula jaguana*) and mullet (*Mugil sp).*Shorebirds, including sanderlings (*Calidris alba*) and ruddy turnstones *(Arenaria interpres)* | USA | Dead fish tissues max 95.7 viscera of sardines, muscle max 0.692 sardine. Brevetoxins in shorebird livers were also confirmed (up to 1.3) | Field Study |

| Species | Country | Maximum NST mg Pb-$TX_2$ /kg (maximum level in bivalves 0.8 mg/kg) | Study type (lab/field/method) |
|---|---|---|---|
| **Other** | | | |
| Dolphin (*Tursiops truncates)* | USA | 0.01 | Field study |
| Manatee *(Trichechus manatus* latirostris) and green sea turtle (*Chelonia mydas*) | USA | | Field study |
| Sponge (*Haliclona tubifera)*, bryozoan *(Bugula neritina),* tunicate *(Styela plicata)* | USA | All 4 accumulated high levels toxin High cell concentrations *K brevis* suppressed clearance rates. | Feeding study |
| Cormorants (*Phalacrocorax auritus*), Brown pelicans (*Pelecanus occidentalis*), Great blue herons (*Ardea herodias*), and Common Loons (*Gavia immer*) | USA | Detected in faeces and blood | Field study |
| Sea turtles, loggerheads, green and Kemp's ridley, Dolphins | USA | 95% sea turtles were positive | Field study |

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