ALGALERT:

A tool to manage Harmful Algal Blooms in New South Wales

UTS:C3
PLANT FUNCTIONAL BIOLOGY & CLIMATE CHANGE CLUSTER

HORNSBY SHIRE COUNCIL

Preface

"Algalert" is a decision support tool developed by the Climate Change Cluster (C3) University of Technology (Sydney) and Hornsby Shire Council which provides coastal managers with the necessary information to monitor and respond to Harmful Algal Blooms (HABs).

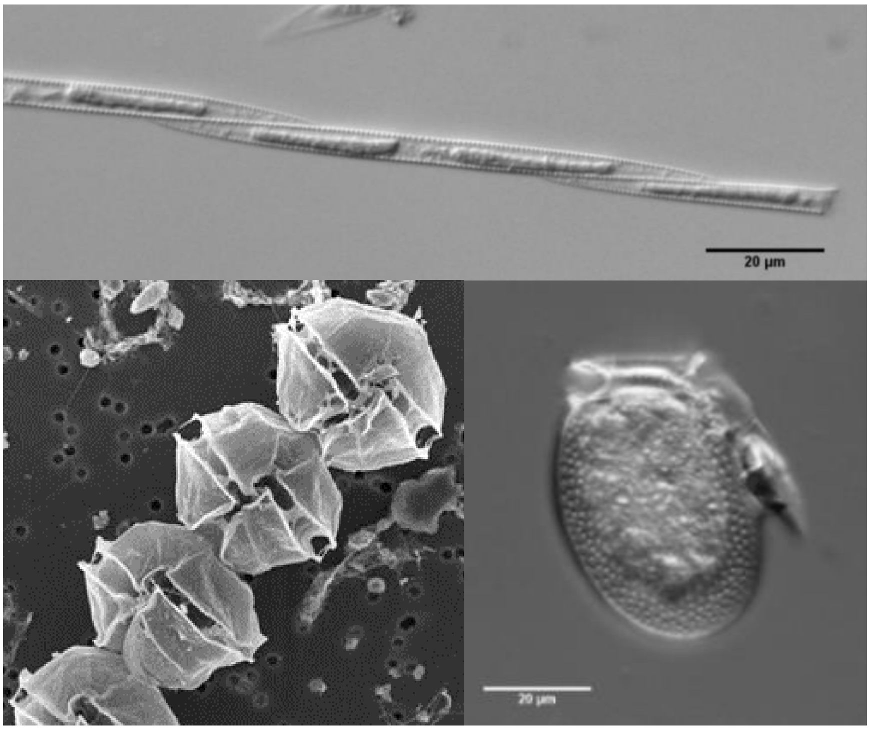
Currently in NSW, when HABs occur, Regional Algal Coordinating Committees are unable to effectively respond. This effectiveness would be improved with access to current, peer reviewed, centralised HAB information. With access to such information, consistent and appropriate public messaging and a clear management response to HABs can be enacted. Improved management of HABs is required as there is general scientific consensus that public health, recreational, commercial, and ecosystem impacts from HABs have increased over the past few decades. Information is critical in managing coastal HABs. "Algalert" provides this knowledge and supports decisions made during bloom periods.

Unlike freshwater blooms, coastal blooms are more complicated, specifically;
(i) low abundance levels can trigger a bloom, (ii) blooms consist of multiple species, (iii) HABs tend to not discolour the waterways (hence public perceive no health problems and disregard warning signs), and (iv) defining the start and end of a bloom is problematic (especially when multiple species are involved).

"Algalert" addresses the complexities of coastal blooms and provides coastal managers with centralised and consistent information to manage HABs in NSW.

This information has been obtained through a collaborative approach. Algal experts from the Climate Change Cluster (C3) UTS, in partnership with Hornsby Shire Council, firstly developed a review of toxic algal species to manage algal blooms. This review involved, (i) a review of the scientific, national and international literature, (ii) a review of the NSW guidelines and policies for management response to marine and freshwater algal blooms including species listed in the New South Wales Food Authority's Marine Biotoxin Management Plan (MBMP, 2015), the National Health and Medical Research Council (NHMRC) Guidelines (2008) and reports generated by the Metropolitan South Coast Regional Algae Co-ordinating Committee and, (iii) extensive data analysis of the 20-year water quality and phytoplankton (Davies et al, 2016) based on monitoring undertaken by Hornsby Shire Council in the Hawkesbury Estuary. All this information is collated in this report.

A Review of Toxic Algal Species towards Improving Management of Toxic Blooms in New South Wales



For

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Executive Summary

Harmful algae and their potential risks are a growing concern for Hornsby Shire Council. This study collates the most up- to-date research on harmful algal species and their potential risks relevant to the Hawkesbury River. It includes microalgal species listed in the New South Wales Food Authority's Marine Biotoxin Management Plan (MBMP, 2015), the National Health and Medical Research Council (NHMRC) Guidelines for Managing Risks in Recreational Waters (2008), and other species identified as being present in the Hawkesbury River as a result of Hornsby Shire Council's monitoring program. Furthermore, species which have been identified in other areas of Australia which might pose a threat to the east coast of Australia and/or the Hawkesbury River as a result of warmer waters shifting south are also included.

This review includes published information and data as well as unpublished information from algal experts.

Harmful Algae

Harmful algae are species of microalgae that can produce toxins that lead to impacts on fisheries, aquaculture, swimming and other recreational uses of estuarine environments. They are a growing phenomenon worldwide, in some cases related to increasing eutrophication and modification of water bodies, as well as marine climate change. In Australia, there is a select, but growing, group of biotoxin-producing estuarine microalgal species that have been documented to cause impacts here in terms of seafood poisonings, the deaths of fish or other marine life, and direct human impacts due to skin exposure or respiratory complaints.

Types of Poisoning/Harmful Effects

The types of poisoning syndromes documented in Australia can be divided into those that only impact humans or the marine food chain through the ingestion of seafood (Amnesic Shellfish Poisoning [ASP], Diarrhetic Shellfish Poisoning [DSP], Neurotoxic Shellfish Poisoning [NSP] and Paralytic Shellfish Poisoning [PSP]), as well as those causing the deaths of marine life, or those causing human skin irritations or breathing difficulties. Marine life that has accumulated levels of toxins does not look or smell differently to other seafood, and therefore, without careful monitoring programs employing microscopy based phytoplankton identification and chemical analyses, it is not possible to detect whether seafood poisoning could occur.

1. <u>Amnesic Shellfish Poisoning</u>

In humans, this type of poisoning is caused by the accumulation of domoic acid. Domoic acid acts as a neurotoxin, crossing into the brain and interfering with nerve signal transmission. Symptoms range from vomiting, nausea, seizures, diarrhea, headaches, dizziness, disorientation, short term memory loss, and permanent brain damage. Species found to contain domoic acid in Australia to date have been:

- *Pseudo-nitzschia australis*
- *Pseudo-nitzschia cuspidata*
- *Pseudo-nitzschia multistriata*

2. Diarrhetic Shellfish Poisoning

In humans, the toxin okadaic acid and its analogs cause 'Diarrhetic Shellfish Poisoning'. Symptoms include diarrhea, nausea, vomiting and cramps. No fatalities from DSP have ever been recorded. The species that has been documented to cause DSP in Australia to date has been:

- *Dinophysis acuminata*

3. Paralytic Shellfish Poisoning

In humans, Paralytic shellfish poisoning is caused by the alkaloid toxin saxitoxin and its analogs. Paralytic shellfish poisoning has caused several illnesses in Australia, which have a range of symptoms including nausea, vomiting, diarrhoea, abdominal pain, tingling or burning lips, gums, tongue, face, neck, arms, legs, and toes. The species that have been documented to produce saxitoxin and its analogs, referred to as the Paralytic Shellfish Toxins, from Australian waters to date are:

- *Alexandrium pacificum*
- *Alexandrium australiense*
- *Alexandrium fundyense*
- *Alexandrium minutum*
- *Gymnodinium catenatum*
- *Dolichospermum sigmoideum* (fresh and brackish water only)

In addition, other types of toxins have been reported to be produced from microalgal species in Australia, including maitotoxin, palytoxin, yessotoxin, and other groups. To date, these generally have not caused a problem with seafood consumption in Australia.

4. Fish killing toxic algal blooms

The deaths of large numbers of fish and other marine/estuarine life, including benthic invertebrates, have occurred in Australia due to blooms of several microalgal species. Sometimes this has occurred in aquaculture ponds or settings, while in other cases it is in bays or estuaries. In NSW, blooms linked to fish kills are a regular phenomenon, with ~20 such instances reported in NSW annually (author obs.) In some cases, the toxins involved in these events have been found and the toxic mechanism is well known. In other cases, the mechanisms of toxicity are less well known. The species that have caused large scale fish kills in Australia to date are:

- *Amphidinium carterae*
- *Karlodinium veneficum*
- *Karenia mikimotoi*
- *Karenia umbella*
- *Takayama pulchella*
- *Chattonella marina*
- *Heterosigma akashiwo*

5. Toxic blooms with direct human effects, causing skin or breathing difficulties

There are a number of species of marine/estuarine and freshwater microalgae that can have direct health impacts on recreational users of the estuary such as those swimming, fishing, boating, and spending time beside the water. These impacts are either through skin contact, which can cause irritation, or through respiratory problems if toxins are inhaled. The species that have been found in Australia and have the potential for causing skin irritations for swimmers are:

- *Amphidinium carterae,*
- *Karlodinium veneficum,*
- *Ostreopsis siamensis,*
- *Ostreopsis ovata,*
- *Moorea producens,*
- *Nodularia spumigena,*
- *Microcystis spp.,* (fresh and brackish water only)
- *Dolichospermum sigmoideum,* (fresh and brackish water only)
- *Noctiluca scintillans*

The species that have the potential to cause respiratory problems, and have been reported anywhere in the world, are:

- *Karenia brevis*
- *Karenia brevisulcata,*
- *Ostreopsis siamensis,*
- *Ostreopsis ovata,*
- *Chattonella marina*

Of these, the two species that have not been as yet found in Australia are *K. brevis* and *K. brevisulcata,* while the other species are present and in some cases, common.

New South Wales Food Authority's Marine Biotoxin Management Plan (MBMP, 2015)

The New South Wales Food Authority (NSWFA) regulates the shellfish industry in NSW in accordance with national and state legislation, which includes the NSW Shellfish Program (NSWSP) and the NSW Biotoxin Management Plan 2015 (NSWBMP, https://foodauthority.nsw.gov.au/_Documents/industry/marine_biotoxin_management_plan.pdf). The NSWBMP aims to ensure the protection of shellfish consumers from the hazards of marine biotoxin poisoning by regular monitoring of phytoplankton and shellfish toxins. An early warning of the potential for contamination of shellfish leads to real-time closures of harvest areas and provides an effective and coordinated response to harmful events.

Phytoplankton Action Limits (PALs) are used to assess the risk of shellfish poisoning events due to the presence of potentially toxic algal species. These action levels are used to trigger additional shellfish flesh testing and/or harvest zone closures (Appendix 1). All harmful species listed in the NSWBMP are included in this review (note some have synonyms).

National Health and Medical Research Council Guidelines for Managing Risks in Recreational Waters (2008)

The NHMRC Guidelines aim to protect the health of humans from threats posed by the recreational use of coastal, estuarine and freshwaters. Harmful algae (whether natural or induced by eutrophication) are included in these threats (Appendix 2). For coastal and estuarine waters cyanobacteria (blue green algae) and algae are listed as potential threats. Each species listed in the guidelines is addressed in this review, with the exception of the following species which have recently been reassessed (since 2008) and shown to be non-toxic eg. *Pfiesteria shumwayae* and *Pfiesteria piscidica*.

Two species of blue-green algae *Oscillatoria nigroviridis* and *Schizothrix calciocda*, both of which can cause swimmers itch, are included in the NHMRC guidelines, but as yet have proven to be rare/not reported in NSW coastal waters. The potential for human health risk should be regarded as for other cyanobacteria (blue-green algae) species in this review.

Other Species which form red tides but are non-toxic
Other microalgal species can cause red tides (water discolorations) but show no toxic effects. Examples of these include the large non-toxic dinoflagellate *Akashiwo sanguinea* (synonym: *Gymnodinium sanguineum*) which has bloomed in NSW estuarine waters including Sydney Harbour, Cooks River/Alexandra Canal (Sydney), Lane Cove River and Berowra Creek (Ajani et al. 2001). *Prorocentrum dentatum*, another dinoflagellate, was responsible for water discolorations in Berowra Creek in May 2003 albeit with no toxic effects. Furthermore, two other dinoflagellates that have bloomed in NSW coastal waters are *Gonyaulax polygramma* ((Sydney Harbour, Bate Bay and Lake Macquarie) and *Scripsiella trochoidea* (Hawkesbury River and Jervis Bay), both with no toxic effects reported (Ajani et al. 2001).

Algal bloom range expansions and climate change.
With ocean warming, tropical species have the potential to extend their range into NSW coastal waters. In particular the dinoflagellate *Pyrodinium bahamense*, a producer of PSP toxins and responsible for >2,000 human illnesses and 100 deaths resulting from the consumption of contaminated shellfish and fish (Hallegraeff and Maclean 1989), is presently confined to tropical, mangrove-fringed coastal waters of the Atlantic and Indo-West Pacific (Hallegraeff 2010). However, the fossil cyst record shows that this species once existed as far south as 32°S, just north of Sydney, and has the potential to extend its range into more southern latitudes in future years.

Similarly, other tropical species belonging to the genus *Gambierdiscus* which produce ciguatoxins (CTXs) and possibly maitotoxins (MTXs) and are the causative species for ciguatera fish poisoning (CFP) in humans, may extend into more southern waters with the 'tropicalisation' of eastern Australia.

Another group of phytoplankton, the haptophytes, have potentially ichthyotoxic (toxic to fish) representatives which, although present in Australian waters, have never bloomed in eastern Australia to date. These include *Chrysochromulina leadbeateri, Phaeocystis globosa*, *Prymnesium parvum* and *Prymnesium polylepis*.

Species identification of bloom of Alexandrium in Calabash Bay, Hawkesbury

In mid December 2014, a bloom occurred in Calabash Bay (site 061) of a species of *Alexandrium* that was identified by light microscopy as *Alexandrium minutum*.

At the time, *Alexandrium minutum* cell numbers were above the cell count threshold for "closure of harvest area pending flesh testing results", at levels > 2000 cells/L, and were also considered to be above the threshold to trigger a recreational warning. A recreational warning was released. Hornsby Council obtained a water sample from the area of the bloom and provided it to UTS for further testing of the species and further identification.

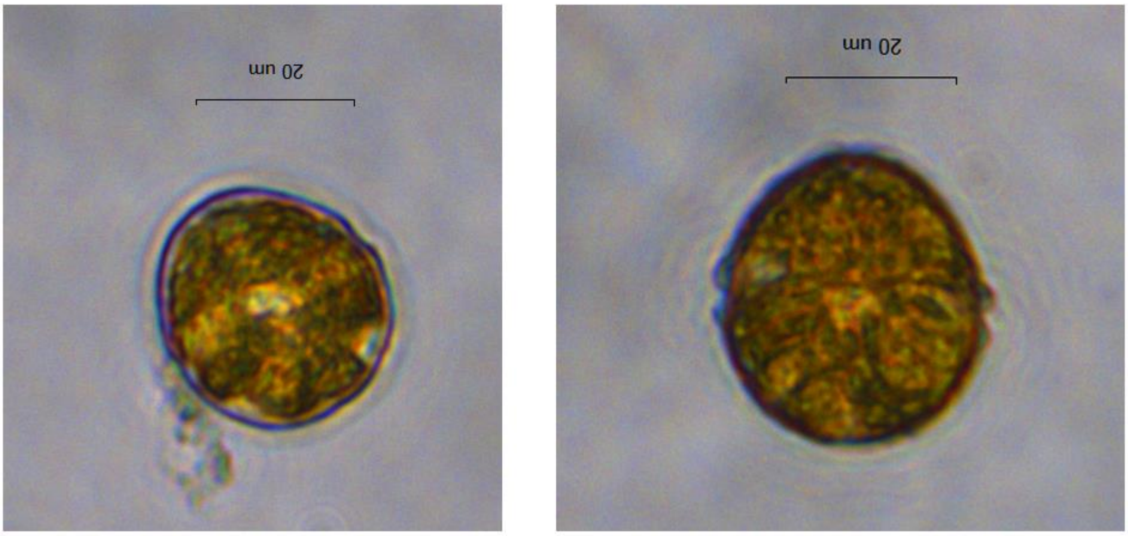
Single cell isolation was performed at UTS on the sample in the following days into sterile GSe media, using a micropipette. One culture, numbered CB1214, grew well and has been kept at UTS, in 20 degrees and a 12/12 light cycle, in GSe media, since that time.

We have tested that culture using multiple methods: toxin analysis using LC/MS/MS to detect the presence of paralytic shellfish toxins, including their many analogs; light microscopy; and genetics based on a region of large subunit ribosomal RNA, a standard 'barcoding' marker region that is commonly used to identify phytoplankton species, and a PCR using sxtA, a region that is specific to a marker for those species that possess paralytic shellfish toxins.

Based on those results, it appears that this species is a new, previously undescribed species of *Alexandrium*, which is a close relative of *Alexandrium minutum* (Figure 1A) However, genetically, in this region, it was found to be 5-10% different to *Alexandrium minutum*, and instead grouped with an undescribed isolate of *Alexandrium* from the USA (Figure 2).

This species did not possess the gene region for *sxtA*. Similarly, it was found to not produce any paralytic shellfish toxins by LC/MS/MS (Figure 1B). UTS will continue to research this strain, as it appears to be a new species, and will therefore describe it as such in the future.

A



B

Target Compounds	RT	Quan Peak	Response	Curve Type	Calculated Conc	Units
C1	N/F	474.035 mz	N/F	Linear	N/F	ng/mL
C2	N/F	474.035 mz	N/F	Linear	N/F	ng/mL
GTX2	N/F	394.079 mz	N/F	Linear	N/F	ng/mL
GTX1	N/F	410.074 mz	N/F	Linear	N/F	ng/mL
dcGTX2	N/F	351.073 mz	N/F	Linear	N/F	ng/mL
dcNEO	N/F	273.131 mz	N/F	Linear	N/F	ng/mL
GTX3	N/F	394.079 mz	N/F	Linear	N/F	ng/mL
GTX4	N/F	410.074 mz	N/F	Linear	N/F	ng/mL
dcGTX3	N/F	351.073 mz	N/F	Linear	N/F	ng/mL
GTX5	N/F	378.084 mz	N/F	Linear	N/F	ng/mL
STX	N/F	300.141 mz	N/F	Linear	N/F	ng/mL
dcSTX	N/F	257.136 mz	N/F	Linear	N/F	ng/mL
NEO	N/F	316.136 mz	N/F	Linear	N/F	ng/mL

Figure 1 A-B. A Light microscope image of a new species of Alexandrium isolated in December 2014 from Calabash Bay (site 061), Hawkesbury River; B. Toxin determination (showing all target compounds) as measured in Alexandrium sp. from from Calabash Bay using LC/MS/MS.

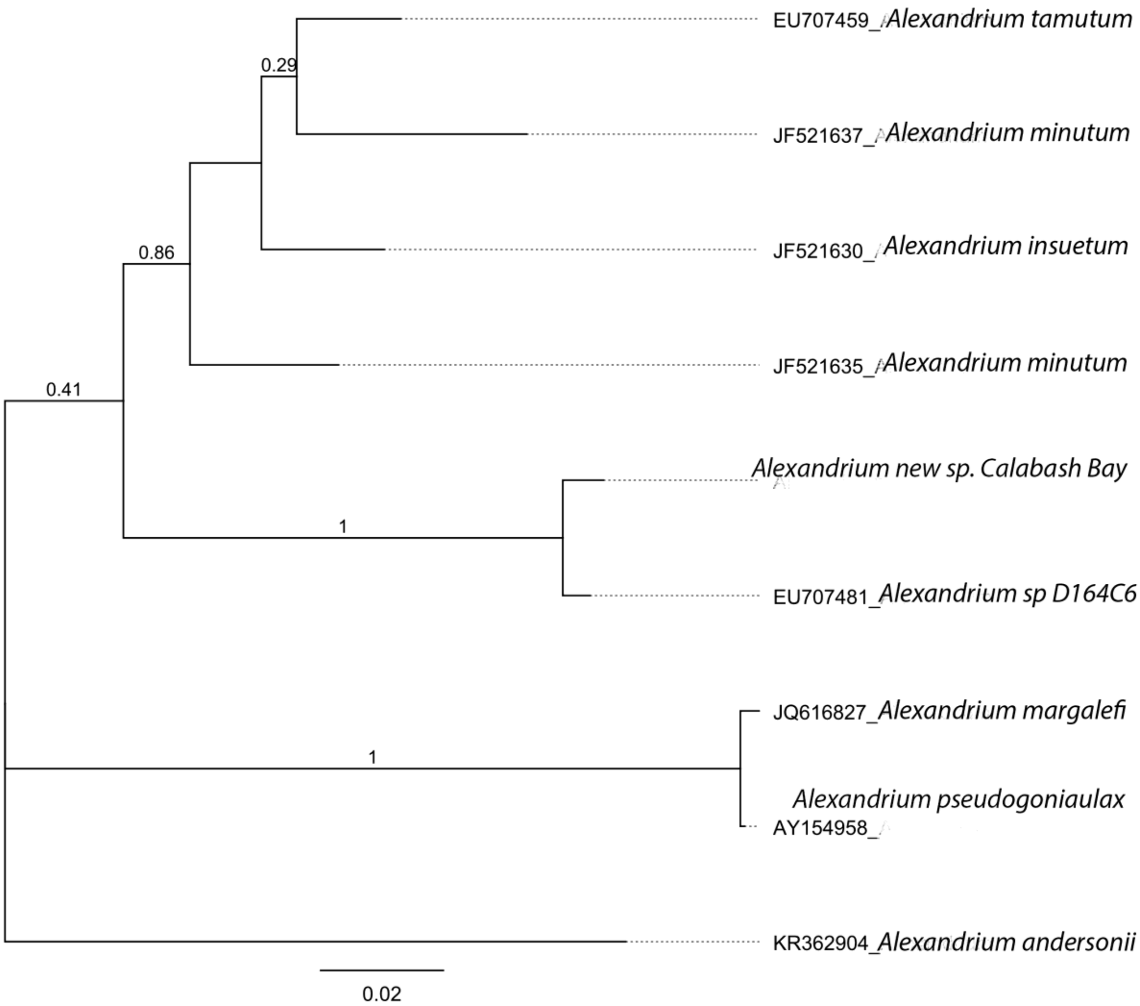


Figure 2. Phylogenetic tree based on the based on a region of large subunit ribosomal RNA showing the new species of Alexandrium from Calabash Bay grouping with and undescribed isolate of *Alexandrium* from the USA (Alexandrium sp D164C6.

Harmful algal species information sheets

The following information sheets list each harmful algal species and information regarding their harmful effects. These sheets are divided into functional groups e.g. diatoms, dinoflagellates, raphidophytes and cyanobacteria [blue-green algae]. A summary list of these species and their reference page is given in Table 1.

The common name and synonym of each species is provided where appropriate, as well as an image (usually microscopic), and biotoxin and toxicity information where known. Also included for each species is information regarding its distribution in Australia (if known), its seasonality (if known) and it's potential to cause a human health problem eg. via consumption of seafood (commercially and recreationally harvested), via aerosol inhalation and/or skin exposure. This risk information is based on previously reported cases or experiments, taken from both local and international literature, as well as anecdotal evidence where possible.

Action limits for each harmful species are also included where possible. If known, the "background" cell concentration of a particular species is given. Other 'limits' provided are based on the NSW Food Authorities Biotoxin Plan (which are in turn based on those levels used internationally and in various states in Australia) and/or cell concentrations at which have been observed to result in toxic events or toxin uptake (anecdotal evidence). These limits should be used to provide an early warning of the potential for marine biotoxin contamination and/or other harmful effects in order to minimize the risk of human illness.

Lastly, this table includes information about the 'impact' of each harmful species and an appropriate warning in regard to each species. For example, if the species causes a visual problem eg. red tide or material washed onto the beach, then "visual amenity" is indicated as an issue. If the species has been reported to cause fish kills, then 'fish kills" are a potential issue. Finally, if the species produces toxins, then it may have an impact on human health (via the consumption of shellfish).

Warnings provide an indication of the effect of each species eg. swimming should be avoided if the species is known to produce skin irritants; shellfish harvest and fishing should be avoided for those species that produce toxins.

These sheets will assist relevant management authorities and the Regional Algal Coordinating Committee (RACC) to assist with the management of algal blooms in NSW waterways. A summary of the process responding to an algal bloom including the role of the different management agencies was developed by Hornsby Shire Council and included in Appendix 3. The algal management response has been summarised in a flow chart that highlights the process in identifying, confirming and responding to an algal bloom in NSW waterways. Appendix 4 summarises the steps that the RACC and relevant management authorities follow once they are alerted of a bloom that exceeds certain thresholds as described in Appendix 3.

Table 1. Harmful species included in this review

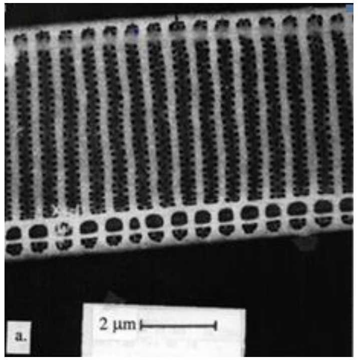
		Reported from Australian waters	Reported from the Hawkesbury River estuary	Page Reference
DIATOMS				
Genus	**Species**			
Pseudo-nitzschia	*australis*	yes	as *P. fraudulenta/australis*	12
Pseudo-nitzschia	*calliantha*	yes	as *P. delicatissima* gp.	13
Pseudo-nitzschia	*cuspidata*	yes	as *P. delicatissima* gp.	14
Pseudo-nitzschia	*fraudulenta*	yes	as *P. fraudulenta/australis*	15
Pseudo-nitzschia	*multiseries*	yes	as *P. pungens/multiseries*	16
Pseudo-nitzschia	*multistriata*	yes	as *P. multistriata*	17
Pseudo-nitzschia	*pungens*	yes	as *P. pungens/multieries*	18
Pseudo-nitzschia	*subpacifica*	yes	as *P. subpacifica/heimii*	19
DINOFLAGELLATES				
Alexandrium	*pacificum*	yes	as *Alexandrium tamarense species complex (A. tamarense, catenella, fundyense*)	20
Alexandrium	*australiense*	yes	*as above*	21
Alexandrium	*fundyense*	yes	*as above*	22
Alexandrium	*minutum*	yes	yes	23
Alexandrium	*ostenfeldii*	yes	yes	24
Amphidinium	*carterae*	yes	as *Amphidinium* sp. ?	25
Dinophysis	*acuminata*	yes	yes	26
Dinophysis	*acuta*	yes	no	27
Dinophysis	*caudata*	yes	yes	28
Dinophysis	*fortii*	yes	yes	29
Dinophysis	*hastata*	yes	no	30
Dinophysis	*tripos*	yes	yes	31
Gambierdiscus	*carpenteri*	yes	no	32
Gonyaulax	*spinifera*	yes	as *Gonylaux* sp.?	33
Gymnodinium	*catenatum*	yes	yes	34
Karlodinium	*veneficum*	yes	as *Karlodinium* sp. ?	35
Karenia	*mikimotoi*	yes	yes	36
Karenia	*brevis*	no	no	37
Karenia	*selliformis*	yes	no	38
Karenia	*brevisulcata*	no	no	39
Karenia	*umbella*	yes	no	40
Lingulodinium	*polyedrum*	yes	yes	41
Ostreopsis	*siamensis*	yes	as *Ostreopsis* sp.?	42
Ostreopsis	*ovata*	yes	as *Ostreopsis* sp.?	43
Noctiluca	*scintillans*	yes	yes	44
Phalachroma	*mitra*	yes	yes	45
Phalachroma	*rotundatum*	yes	yes	46
Prorocentrum	*mimimum*	yes	yes	47
Prorocentrum	*lima*	yes	no	48
Prorocentrum	*rhathymum*	yes	no	49
Takayama	*pulchella*	yes	yes	50
CHLOROMONADS (RAPHIDOPHYTES)				
Chattonella	*marina*	yes	yes	51
Heterosigma	*akashiwo*	yes	yes	52
Fibrocapsa	*japonica*	yes	yes	53

		Reported from Australian waters	Reported from the Hawkesbury River estuary	Page Reference
CYANOBACTERIA (BLUE-GREENS)				
Dolichospermum	*sigmoideum*	yes	yes	54
Moorea	*producens*	yes	as *Lyngbya* sp.?	55
Microcystis	*weisenbergii,*	yes	yes	56
	aeruginosa,		yes	
	flosaquae		as *Microcystis* sp. ?	
Nodularia	*spumigena*	yes	yes	57
Trichodesmium	*erythraeum*	yes	yes	58

Diatoms (Bacillariophyceae)

Species

Pseudo-nitzschia australis
(seriata gp.*)*

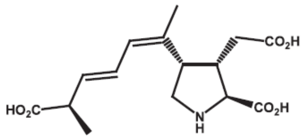


Lapworth et al. 2001

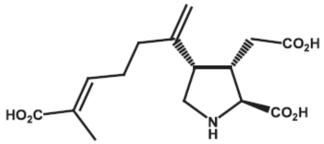
Biotoxin

Domoic Acid (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).

Domoic Acid



Isodomoic Acid C



Toxicity

Fritz et al. (1992) noted this species is the likely source of domoic acid from a bloom of which occurred in Monterey Bay, California, September 1991. Garrison (1992) confirmed the presence of domoic acid in single clone isolates of *P. australis* from Monterey Bay, California. Maximum concentrations were reported as 37 and 12 pg DA cell^{-1}. Rhodes et al. (1997, Harmful Algae Newsletter) suggests domoic acid found in scallops was linked to high abundance of *P. australis* in Northland, New Zealand.

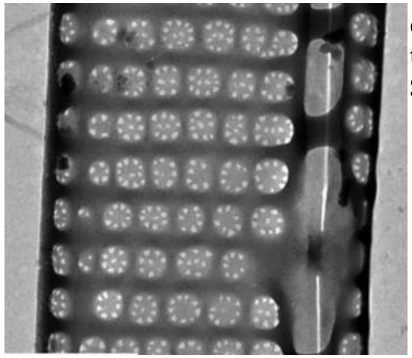
Lapworth et al. (2000) preliminary analysis using ELISA confirmed significant levels of domoic acid in three cultures of *P. australis* tested from Tasmanian waters (1-500 ng/ml DA). Holland et al. (2005) and Rhodes et al. (2006, Harmful Algae Newsletter) both demonstrated the production of isodomoic acid-C (isoDA-C) in *P. australis*.

McKibben et al. (2015) recommend a water column concentration of particulate domoic acid greater than 103 ng L-1 can be used as a threshold for early-warning of shellfish DA toxicity.

Known Distribution in Australia	Tasmania, Victoria, New South Wales (National Reference Station, Port Hacking 100m) (Lapworth et al. 2001; Ajani et al. 2001a)
Known Seasonality	Enumerated as *P. fraudulenta/australis,* this group shows minimal abundance in autumn/winter and maximum in spring (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans is only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species

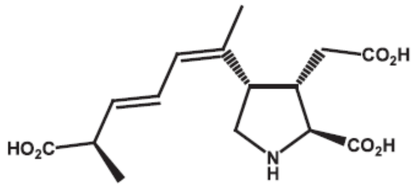
Pseudo-nitzschia calliantha
(delicatissima gp.*)*



Ajani et al. 2013a

Biotoxin

Domoic Acid (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).



Lelong et al. 2012

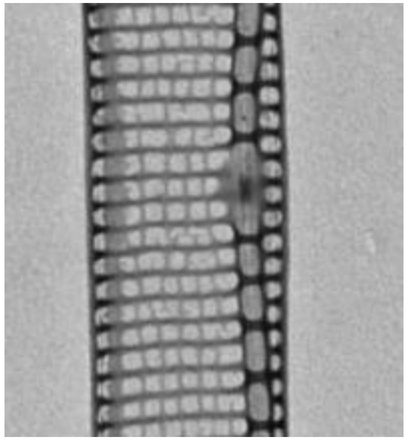
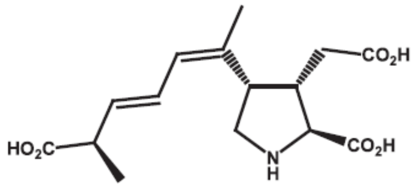
Toxicity

In 2003, Lundholm et al. redescribed three species previously delineated as *Pseudo-nitzschia pseudodelicatissima – P. calliantha, P. caciantha* and *P. cuspidata*.

Previous work of Lapworth et al. (2001) on five strains of *P. pseudodelicatissima* found one produced detectable levels of domoic acid (1 $ng.ml^{-1}$).

Seven strains of *P. calliantha* isolated from NSW estuaries in 2011 (Wallis Lake, Wonboyn River, Wagonga Inlet and Tuross Lake) and the Derwent River (Tasmania) were shown to be non toxic (Ajani et al. 2013a). Futhremore, those strains identified previously as *P. pseudodelicatissima* in Hallegraeff 1994 and Lapworth et al. (2001) were determined to be *P. calliantha*.

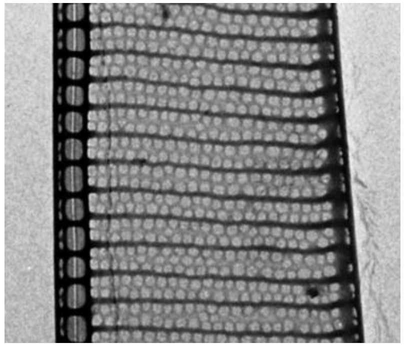
Known Distribution in Australia	Tasmania (Derwent River), Victoria, New South Wales (Wallis Lake, Wonboyn River, Wagonga Inlet, Tuross Lake, Botany Bay) (Jameson & Hallegraeff 2010; Ajani et al. 2013a)
Known Seasonality	Enumerated in the *P. delicatissima* group, this group shows maximum abundance spring (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans is only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Pseudo-nitzschia cuspidata *(delicatissima* gp.*)*  Ajani et al. 2013a	*Domoic Acid* (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).  Lelong et al. 2012	In 2003, Lundholm et al. redescribed three species previously delineated as *Pseudo-nitzschia pseudodelicatissima* – *P. calliantha, P. caciantha* and *P. cuspidata*. Bill et al. 2005 confirmed domoic acid in *Pseudo-nitzschia cuspidata* from Washington State coastal waters. Shellfish from Wagonga Inlet, south eastern Australia, detected positive for DA during a *Pseudo-nitzschia cuspidata* bloom on 25/5/10. Ajani et al. (2013a) confirmed toxicity in *P. cuspidata* from Lake Merimbula collected January 2012 (25.4 pg DA per cell^{-1}) and from 4.3 pg DA per cell in cells isolated offshore from Port Hacking in March 2012.

Known Distribution in Australia	New South Wales (Bondi Beach, Wagonga Inlet, Patonga Creek, Merimbula Lake) (as strain *Sydney 1* Lundholm et al. 2003, Ajani et al. 2013a)
Known Seasonality	Enumerated in the *P. delicatissima* group, this group shows maximum abundance spring (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species

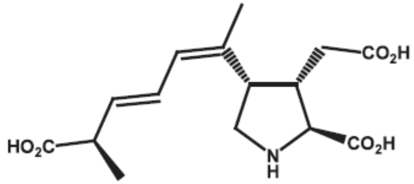
Pseudo-nitzschia fraudulenta
(*seriata* gp.)



Ajani et al. 2013a

Biotoxin

Domoic Acid (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).



Lelong et al. 2012

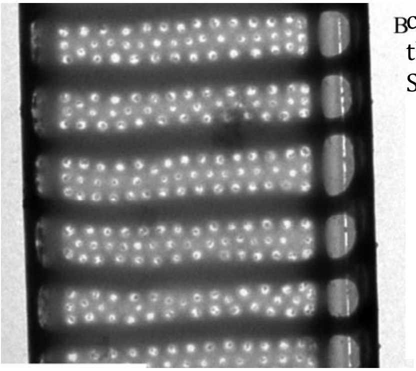
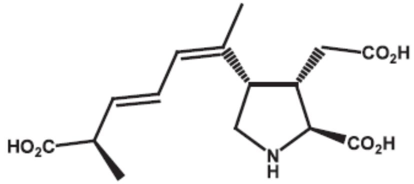
Toxicity

Lapworth et al. (2001) found no DA present in Tasmanian strains of *P. fraudulenta*.

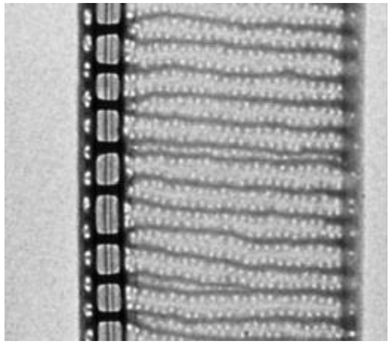
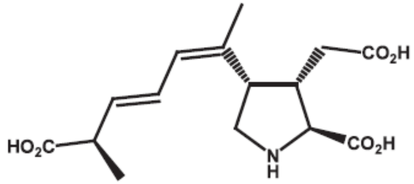
Rhodes et al. (1997) using DA immunoassays found *P. fraudulenta* from New Zealand waters produced domoic acid.

Two strains isolated from NSW coastal waters in 2011/12 from Meribula and Port Hacking (25m) were both revealed to be non-toxic (Ajani et al. 2013a).

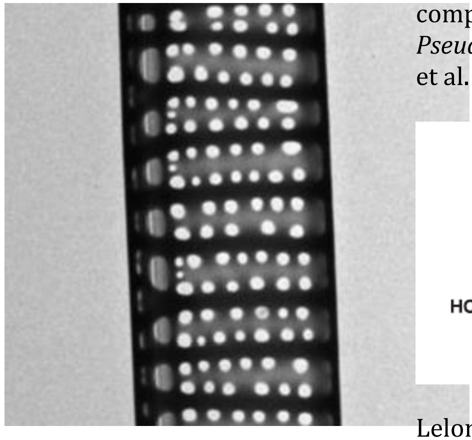
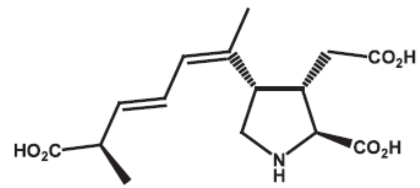
Known Distribution in Australia	Victoria (Port Phillip Bay), New South Wales (Port Hacking 25m and Berowra Creek), North Western Australia, Gulf of Carpentaria and Queensland (Lapworth et al. 2001, Jameson & Hallegraeff 2010, Ajani et al. 2013a)
Known Seasonality	Enumerated as *P. fraudulenta/australis,* this group shows minimal abundance in autumn/winter and maximum in spring (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Pseudo-nitzschia multiseries (*seriata* gp.)  Ajani et al. 2013a	*Domoic Acid* (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).  Lelong et al. 2012	*P. multiseries* was the first algal species found to produce the neurotoxin domoic acid. This was discovered in 1987 after which the first amnesic shellfish poisoning (ASP) event occurred. Three people died and hundreds of people were ill after the consumption of blue mussels (*Mytilus edulis*) (Bates et al. 1989). The toxin was traced to a bloom of *P. multiseries* upon which the mussels had been feeding. This species has been found to be a consistent producer of DA in all strains tested throughout the world (Lelong et al. 2012) yet isolates from south-eastern Australia (Coogee Beach, collected 2011) are the first nontoxic strains reported in the world (Ajani et al. 2013a).

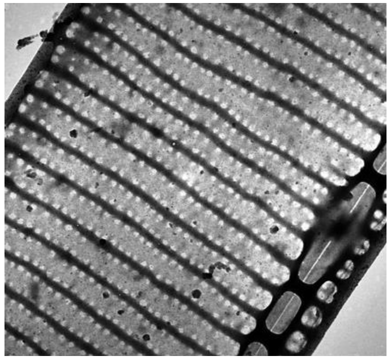
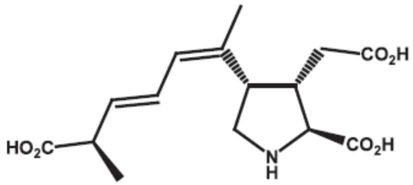
Known Distribution in Australia	New South Wales (Berowra Creek, Port Hacking and Coogee Beach (Lapworth et al. 2001, Jameson & Hallegraeff 2010, Ajani et al. 2013a)
Known Seasonality	Enumerated as *P. pungens/multiseries,* this group shows minimal abundance across all seasons with the exception of the Hawkesbury River, which reached maximum cell densities in autumn (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Pseudo-nitzschia multistriata (*delicatissima* gp.)  Ajani et al. 2013a	*Domoic Acid* (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).  Lelong et al. 2012	All strains of *P. multistriata* tested for DA from Australian waters have been positive for DA with toxin concentration ranging from <1 to 11 DA per cell. *Pseudo-nitzschia multistriata* strains (PH25B-191011, PH25C-191011, PH25D-191011) were isolated from Port Hacking in October 2011 and Wapengo Lake (strain WAPB-311011) also in October 2011 (Ajani et al. 2013a).

Known Distribution in Australia	New South Wales (Port Hacking and Berowra Creek) (Jameson & Hallegraeff 2010, Ajani et al. 2013a)
Known Seasonality	Enumerated as the *P. delicatissima* group, this group shows minimal abundance in autumn/winter and maximum in spring in Wallis Lake and Wagonga Inlet, while is present all year round in the Hawkesbury with a slight increas in autumn (Ajani et al. 2013a).
Potential for human health risk	Toxicty to humans only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

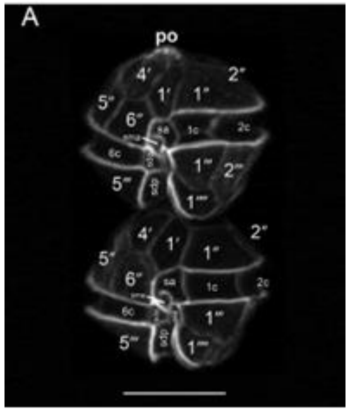
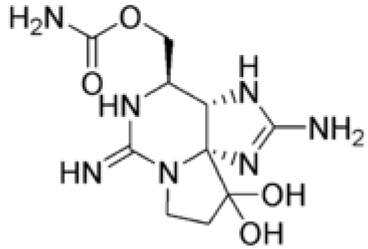
Species	Biotoxin	Toxicity
Pseudo-nitzschia pungens *(seriata* gp.*)*  Ajani et al. 2013a	*Domoic Acid* (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).  Lelong et al. 2012	This species is commonly seen in the Hawkesbury River, but isolates from Australian coastal waters (Dromana Beach, VIC and Wonboyn Lake, NSW) have not yet shown toxicity (Ajani et al. 2013a).

Known Distribution in Australia	Tasmania (Derwent River), Victoria (Dromana Beach), New South Wales (Wonboyn Lake, Hawkesbury River), Western Australia (Swan River) (Jameson & Hallegraeff 2010, Ajani et al. 2013a)
Known Seasonality	Enumerated as *P. pungens/multiseries,* this group shows minimal abundance across all seasons with the exception of the Hawkesbury River, which reached maximum cell densities in autumn (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

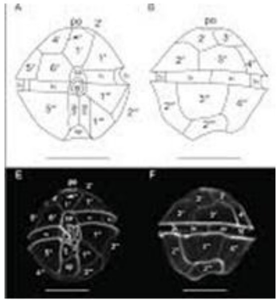
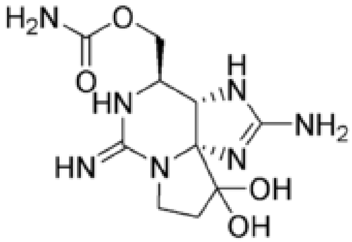
Species	Biotoxin	Toxicity
Pseudo-nitzschia subpacifica *(seriata* gp.*)*  Moschandreou et al. 2012	*Domoic Acid* (DA) and its isomers are neurotoxins causing Amnesic Shellfish Poisoning. DA does not accumulate in the water column because it is produced in low quantities compared to the ocean, and/or it sinks while still within the *Pseudo-nitzschia* cells (Sekula-Wood et al. 2009, 2011; Silver et al. 2010).  Lelong et al. 2012	While *P. subpacifica* was first identified in Australian waters by Hallegraeff (1994), and observed in environmental samples by Ajani et al. (2013), this taxon remains poorly defined and warrants further investigation in eastern Australia. Strains of the species from Gulf of Maine, North-West Atlantic, have been confirmed to produce domoic acid at the levels 0.06-1.1 ng/ml (Fernandes et al. 2014). No Australian strains have been tested for the presence of domoic acid.

Known Distribution in Australia	Tasmania (Derwent River), New South Wales (Jameson & Hallegraeff 2010, Ajani et al. 2013a) and South Australia (Lapworth et al. 2001)
Known Seasonality	Enumerated as *P. heimii/subpacifica* this group was generally low across all seasons with the exception of Wallis Lake in summer (Ajani et al. 2013a)
Potential for human health risk	Toxicty to humans only via consumption of seafood (shellfish, crabs, squid, octopus, fish). There are no cases of domoic acid inhalation and no experiments to evaluate aerosol exposure to this toxin.
Action Levels	**Usually present <5 x 10^4 cells L^{-1}; seafood toxicity testing >5 x 10^4 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Dinoflagellates (Dinophyceae)

Species	Biotoxin	Toxicity
Alexandrium pacificum (formerly known as *Alexandrium catenella,* and *Alexandrium tamarense Group IV)*  John et al. 2014	*Saxitoxin* (SXT) and its isomers, including the gonyautoxins and neosaxitoxin, are neurotoxins causing Paralytic Shellfish Poisoning (PSP).  The main toxin components of *A. pacificum* cultures from Australia are GTX 1,4, STX and the C toxins (Murray et al. 2011, Negri et al. 2003). PSP acts as a neurotoxin, crossing into the brain and interfering with nerve signal transmission.	The species that were formerly known as the *Alexandrium tamarense* species complex (*A. tamarense, catenella, fundyense*) are morphologically highly similar to one another, and genetic methods are normally required to confirm their identities. This species is the main source of PSP toxins in NSW and Victorian marine waters, and has been found at several sites on the east coast of Tasmania (Bolch and de Salas 2007). Blooms of *Alexandrium pacificum* have accounted for more than 50% of algal related shellfish aquaculture harvesting closures since 2005 in NSW (Farrell et al. 2013). While this species has been identified at all NSW estuaries south of the Richmond River, the estuaries that have generally been most impacted by blooms of this species have been: the Georges River, the Hawkesbury River, and Brisbane water (Farrell et al. 2013). Maximum abundances of this species in NSW have been in the order of 10-20,000 cells/L. In the Hawkesbury River, the greatest concentrations have been found between September-November, and highest at Station 34 (Farrell et al. 2013). As this species can form cysts, which can persist in the sediment for long periods of time, it is common that blooms recur in similar regions.

Known Distribution in Australia	Tasmania (Triabunna, east coast), Victoria , New South Wales (widespread) (Bolch and de Salas, 2007, Farrell et al. 2013, Hallegraeff et al. 1991)
Known Seasonality	Greatest abundances of *Alexandrium pacificum* in NSW are during Spring and Summer.
Potential for human health risk	Toxicty to humans is generally via consumption of seafood (shellfish, crabs, squid, octopus, fish).
Action Levels	**Levels above 200 cells/L can be associated with toxin uptake.**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

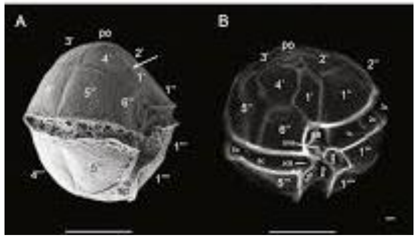
Species	Biotoxin	Toxicity
Alexandrium australiense (formerly known as *Alexandrium tamarense* Group V genotype) John et al. 2014 	*Saxitoxin* (SXT) and its isomers, including the gonyautoxins and neosaxitoxin, are neurotoxins causing Paralytic Shellfish Poisoning (PSP).  The main toxin components of *A. australiense* cultures from Australia are GTX5 and STX, which is a comparatively unusual toxin profile compared to other *Alexandrium* species (Murray et al.2012).	The species that were formerly known as part of the *Alexandrium tamarense* species complex (*A. tamarense, catenella, fundyense*) are morphologically highly similar to one another, and genetics is normally required to confirm their identities. This distribution of this species in the Australasian region is not well known, due to its morphological similarity to other strains of the *Alexandrium tamarense* "species complex" (Murray et al. 2012, John et al. 2014). Cultures have been isolated from Tasmanian and South Australian waters. Four strains have been tested for PSTs, of which, a strain from Tasmania showed detectable toxin production with standard LCMS methods. Very low levels of STXs may be produced by a further strain ATBB01/CS298 from Tasmania, as it showed activity with the sensitive saxiphilin assay (Scholin et al., 1994; Negri et al., 2003). Incidents of shellfish toxicity associated with this species appear rare and unconfirmed. A possible incident in NSW in the Hastings River in 2010 may be associated with this species. As this species can form cysts, which can persist in the sediment for long periods of time, it may recur in similar regions.

Known Distribution in Australia	Tasmania, South Australia, NSW? (Farrell et al. 2013, Hallegraeff et al. 1991, Murray et al. 2012)
Known Seasonality	Seasonality is not known.
Potential for human health risk	Toxicty to humans is generally via consumption of seafood (shellfish, crabs, squid, octopus, fish).
Action Levels	**Levels above 200 cells/L can be associated with toxin uptake.**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species

Alexandrium fundyense

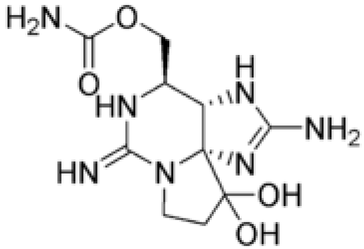
(formerly known as Alexandrium tamarense, A. catenella, and Alexandrium tamarense Group I)



John et al. 2014

Biotoxin

Saxitoxin (SXT) and its isomers, including the gonyautoxins and neosaxitoxin, are neurotoxins causing Paralytic Shellfish Poisoning (PSP).



The main toxin components of *A. fundyense* cultures from Australia have been found to be C1/2 and GTX1/4, low proportions of NEO, C3/4, and traces of GTX2/3 and dcGTX2/3, with an 8-fold variation in STX content (8-65 fmol cell $^{-1}$) among strains (Bolch et al. 2014).

Toxicity

The species that were formerly known as part of the *Alexandrium tamarense* species complex (*A. tamarense, catenella, fundyense*) are morphologically highly similar to one another, and genetics is normally required to confirm their identities.

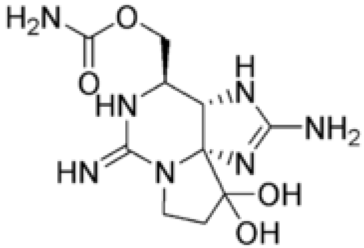
In 2012, STXs were detected in shellfish on Tasmania's east coast, resulting in harvest closures of mussels, oysters, scallops, rock lobster and abalone over a period of six months along 350 km of coastline, with total economic losses estimated at $23M (Bolch et al. 2014). ~20 cultures were established and DNA sequence analysis confirmed all isolates as *A.fundyense*, not previously known from Australasia, and confirmed that all isolates produced PSTs.

As yet, this species has only been found in Tasmania in Australia. It is a common species in North and South America, and is also known from European waters (Sephton et al. 2007, Martin et al. 2006). It can be associated with fish kills or with toxin uptake in fish in these regions (Sephton et al. 2007, Martin et al. 2006).

Known Distribution in Australia	East coast of Tasmania (Bolch et al. 2014)
Known Seasonality	In Tasmania, this species appears to bloom in Winter/Spring (unpublished data)
Potential for human health risk	Toxicty to humans is generally via consumption of seafood (shellfish, crabs, squid, octopus, fish).
Action Levels	**Levels above 200 cells/L can be associated with toxin uptake.**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Alexandrium minutum	*Saxitoxin* (SXT) and its isomers, including the gonyautoxins and neosaxitoxin, are neurotoxins causing Paralytic Shellfish Poisoning (PSP).	*Alexandrium minutum* has been the causative species of two STX toxin events resulting in shellfish harvest closures in NSW, in the Hawkesbury River during 2007 (March) and at Port Stephens in 2009 (December) (Farrell et al. 2013). It is distributed widely in NSW, from the Tweed River in the north of the state to Wonboyn Lake in southern NSW (Farrell et al. 2013), although generally in low abundances. Maximum cell concentrations occurred in the late summer, early autumn months, and were relatively low, in the order of 10^3 cells/L.

Hansen et al. 2003



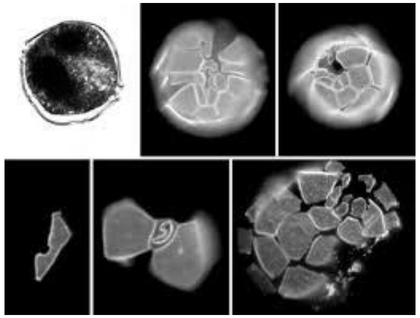
The main toxin components of *A. minutum* cultures from Australia have been found to be GTX 1,4, with a small proportion of STX (Negri et al. 2003, Farrell et al. 2015).

A.minutum has also occurred in South Australian estuaries, and bloomed in the Port River in South Australia in 1986 and 1987 (Hallegraeff et al., 1988). *A. minutum* has also been reported from Tasmania (Bolch et al. 1991, Hallegraeff et al. 1991).
It is a common species worldwide, it can become extremely abundant in some locations, and has been responsible for large scale regular shellfish aquaculture closures in European countries, particularly in France and Ireland (Touzet et al. 2007).

Known Distribution in Australia	NSW, SA, Tasmania (Farrell et al. 2013, Hallegraeff et al. 1991, Bolch et al. 1991)
Known Seasonality	Late Summer, early Autumn in NSW.
Potential for human health risk	Toxicty to humans is generally via consumption of seafood (shellfish, crabs, squid, octopus, fish).
Action Levels	**Levels above 200 cells/L can be associated with toxin uptake.**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species

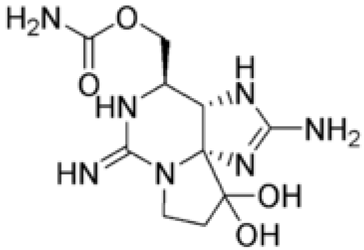
Alexandrium ostenfeldii



Mackenzie et al. 2004

Biotoxin

Saxitoxin (SXT) and its isomers, including the gonyautoxins and neosaxitoxin, are neurotoxins causing Paralytic Shellfish Poisoning (PST).



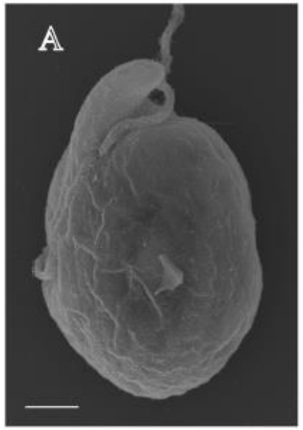
No culture of *A. ostenfeldii* from Australia has yet been tested for toxicity, so the precise toxins produced by local strains are not yet known. Internationally, strains have been found which produce STXs, but non-STX producers have also been found.

Toxicity

Alexandrium ostenfeldii is distributed all along the NSW coastline from the Bellinger/Nambucca River to Wonboyn Inlet, and found throughout the year, but at relatively low abundances ~10^2 cells L^{-1} (Farrell et al. 2013). *Alexandrium ostenfeldii* was the only species (150 cells L^{-1}) identified during a positive PSP event in Twofold Bay in NSW in 2007. However, as no culture has been established, the toxicity of local strains of this species have not been verified.

Internationally, this species produces large scale blooms in the Baltic Sea, and the Netherlands, that have impacted shellfish aquaculture industries (Kremp et al. 2007) .

Known Distribution in Australia	Tasmania (unpublished data), NSW coastline (Farrell et al. 2013)
Potential for human health risk	Toxicity to humans is generally via consumption of seafood (shellfish, crabs, squid, octopus, fish).
Known Seasonality	Found throughout the year, particularly in Spring/Summer months
Action Levels	**Levels above 200 cells/L can be associated with toxin uptake.**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Amphidinium carterae  Murray et al. 2012	The biotoxins involved in blooms of *Amphidinium carterae* are incompletely known. Many different types of toxic compounds are produced by strains of *Amphidinium carterae* and related species (summarised in Murray et al. 2012), including macrolides, short polyketides, and long chain polyketides. Some commonly produced substances are amphidinols and amphidinolides. A compound called luteophanol, chemically similar to amphidinol, was found to be produced by the strain of *Amphidnium carterae* during the Sydney bloom in 2012 (Murray et al. 2015). This compounds caused a loss of viability in assays with fish gill cells.	*Amphidinium carterae* has formed a very dense bloom at the shallow sandy intermittently open coastal lagoon, Curl Curl on the northern beaches of Sydney, NSW (1.8×10^8 cells L^{-1}) (Murray et al. 2015). This bloom caused visible water discolouration. This bloom co-occurred with the deaths of >300 individuals of three different species of fish. The opening of the lagoon to the ocean, as well as localized high nutrient levels, preceded the observations of very high cell numbers. *A. carterae* is usually sediment-dwelling, but temporarily became abundant throughout the water column in this shallow (<2 m) sandy habitat. Histopathological results showed that the *Anguilla reinhardtii* individuals examined had damage to epithelial and gill epithelial cells. Fish kills due to this species have also been reported from Israel and Portugal, at similarly high cell abundance levels (Murray et al. 2015).

Known Distribution in Australia	Not known, but likely to be widespread.
Potential for human health risk	May cause fish kills, and elevated pH that may cause skin irritations
Known Seasonality	Present year round at the sites where it has been monitored
Action Levels	**Not set, and incompletely known. Fish kills in NSW and overseas occurred at levels of > 10^6 cells L^{-1}**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☐ Swimming ☐ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Dinophysis acuminata  Ajani 2014	Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004a, b).	This species is main agent of DSP events around the world – Europe, Japan, New Zealand and north eastern and northwestern America. DSP toxins in oysters and mussels first linked to *D. acuminata* (Pitcher, et al. 2011). Some strains appear to produce only PTX, others only OA, others DTX1 and PTX2 or a mixture of OA, DTXs and PTXs (IOC-UNESCO and references therein http://www.marinespecies.org/hab/). *Dinophysis acuminata* blooms have been linked with thermally stratified temperate waters (Reguera et al. 2014 and references therein).

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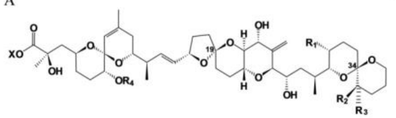
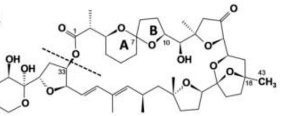


Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

B



Reguera et al. 2014

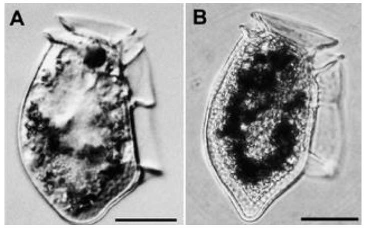
D. acuminata has also been responsible for three major DSP events in Australia to date. In 1997 *D. acuminata* (and *D. tripos*) was implicated in the contamination of pipis (*Plebidonax deltoides* Lamarck 1818) in New South Wales (NSW) (Quaine et al. 1997) in which 102 people were affected, and 56 cases of gastroenteritis reported. In March 1998 a second outbreak was reported in which 20 cases of DSP poisoning were reported (Mackenzie et al. 2002). In December 2003, another *D. acuminata* bloom was detected in the Eyre Peninsula, South Australia (SA) (Madigan et al. 2006). Statistical modelling of *D. acuminata* blooms in the Hawkesbury River over the period 2003-2014 reveal they are linked to season (spring), thermal stratification (increasing) and nutrients (decrease in Redfield ratio) (Ajani et al. submitted).

Despite its importance, many aspects of *Dinophysis* (life history, toxicity, genetic diversity, and population heterogeneity) have remained undiscovered until very recently. This has been due to an inability to successfully maintain cultures of these organisms in the laboratory (Sampayo et al. 1993, Nishitani et al. 2003).

Known Distribution in Australia	Common in Australian coastal waters but rarely abundant; Derwent River, Tasmania (Hallegraeff 2015); New South Wales coastal waters including Hawkesbury River (Ajani et al. 2001a, 2011, 2013b and 2016 submitted)
Known Seasonality	Highest abundance in Hawkesbury seen in spring (max. abundance 4,500 cells l^{-1}) (Ajani et al. 2016 submitted)
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually present <1 x 10^3 cells L^{-1}; seafood toxicity testing >1 x 10^3 cells L^{-1} (Reguera et al. 2014)**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity

Dinophysis acuta



MacKenzie et al. 2005

Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTXs), and (ii) pectenotoxins (PTXs). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004).

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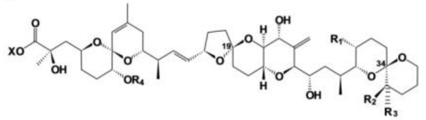

Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

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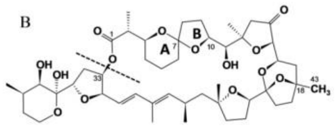


Reguera et al. 2014

D. acuta is a causative species for DSP (second after *D. acuminata)* in Europe (in particular Norway, Sweden, Ireland, Portugal and Spain) and New Zealand.

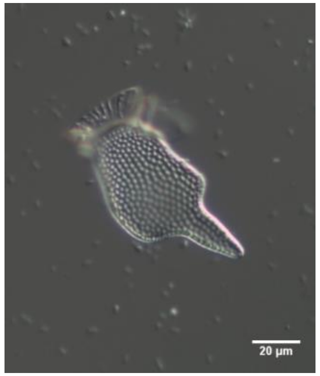
There have been no reports of DSP as a result of *D. acuta* in Australian waters to date.

Most strains produce diarrhetic shellfish toxins (OA, DTX1 and/or DTX2) and pectenotoxins (PTX2 and PTX11 or PTX12) but some strains with only PTX2 may occur (IOC-UNESCO and references therein http://www.marinespecies.org/hab/)

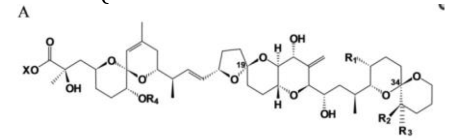
Dinophysis acuta blooms have been linked with thermally stratified temperate waters (Reguera et al. 2014 and references therein) at approximately ~10-20m (Farrell et al. 2009).

Known Distribution in Australia	Relatively rare in Australian coastal waters; New South Wales coastal waters (Ajani et al. 2001a. 2011 & 2013b)
Known Seasonality	No detailed seasonal abundance data is available for this species in Australian waters however NSW wide phytoplankton monitoring data suggest presence in late summer and spring (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Dinophysis caudata	Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004). OA, DTX1 and PTX2 have been detected in *D. caudata* (IOC UNESCO and references therein)	DSP outbreaks associated with blooms of *D. caudata*, which often co-occur with other *Dinophysis* species, have been reported from Europe, America and Asia.

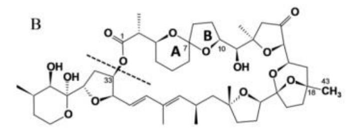


Ajani 2014



Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols



Reguera et al. 2014

Santhanam & Srinivasan (1996) report on the impact of a *Dinophysis caudata* bloom on the hydrography and fishery potentials of Tuticorin Bay, South India.

Statistical modelling of *D. caudata* blooms in the Hawkesbury River over the period 2003-2014 show that blooms are linked to nutrients (decrease in Redfield ratio)), salinity (~20ppt) and a reduction in dissolved oxygen (Ajani et al. 2016 submitted). Other studies around the world have shown that *D. caudata* sometimes accompanies *D. acuta* blooms during stratification periods (Reguera et al. 2014 and references therein).

There have been no reports of DSP as a result of *D. caudata* in Australian waters to date.

Known Distribution in Australia	Common in Australian coastal waters and sometimes abundant; New South Wales coastal waters including Hawkesbury River (Ajani et al. 2001a, 2011, 2013b & 2016 submitted)
Known Seasonality	Highest abundance in Hawkesbury seen in the summer to autumn (max. 12,000 cells l^{-1}) (Ajani et al. 2016 submitted)
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually present <500 cells L^{-1}; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Dinophysis fortii	Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004).	*Dinophysis fortii* was found to be the causative organism for mussel toxicity in Japan in 1980, whereby the toxin received its name dinophysistoxin, and the term "diarrhetic shellfish poisoning" proposed due to the major symptom in humans of diarrhea (Yasumoto et al. 1980)

Ajani 2014

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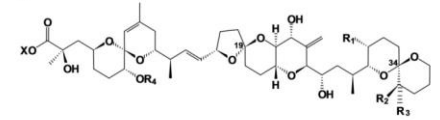

Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

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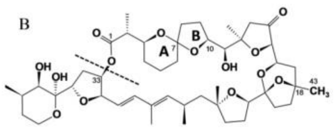


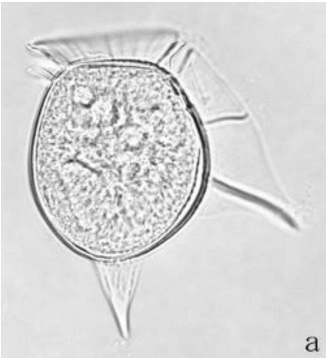
Reguera et al. 2014

Producer of okadaic acid (OA), dinophysis toxins (DTX1) and pectenotoxins (PTX2) (Suzuki et al. 1996, Draisci et al. 1996)

Low levels OA and DTX1 have been detected in wild Tasmanian mussels, but there have been no reports of DSP as a result of *D. fortii* in Australian waters to date (Hallegraeff 2015).

Known Distribution in Australia	Common in Australian waters but rarely abundant; mixed blooms with *D. acuminata* Oct – Feb in Derwent River, Tasmania (Hallegraeff 2015); New South Wales coastal waters including Hawkesbury River (Ajani et al. 2001a, 2011, 2013b and 2016 submitted).
Known Seasonality	No detailed seasonal abundance data is available for this species in Australian waters however NSW wide phytoplankton monitoring data suggest presence in late summer and spring (unpublished data). Seen rarely in Hawkesbury River (Ajani et al. submitted).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Relatively rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Dinophysis hastata	Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004). *D. hastata* produces okadaic acid and dinophysistoxins (Todd 2011).	There have been no reports of DSP as a result of *D. hastata* in Australian waters to date.



http://tapapu.org/

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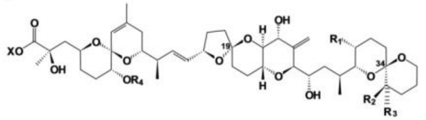

Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

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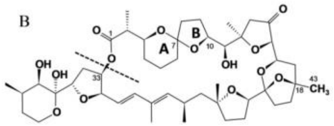
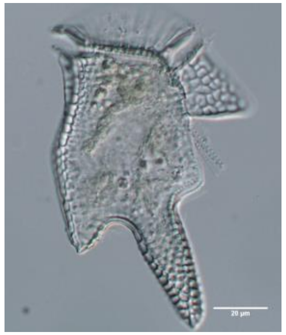


Reguera et al. 2014

Known Distribution in Australia	Relatively rare in Australian coastal waters; New South Wales coastal waters including Brisbane waters and Hawkesbury River (Ajani et al. 2013b)
Known Seasonality	No detailed seasonal abundance data is available for this species in Australian waters however NSW wide phytoplankton monitoring data suggest presence in summer only (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity

Dinophysis tripos



Ajani 2014

Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004).

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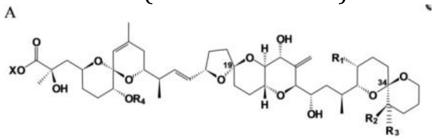

Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

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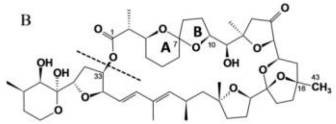


Reguera et al. 2014

DTX1 was detected using HPLC-FD analysis of picked *D. tripos* cells (Lee et al. 1989).

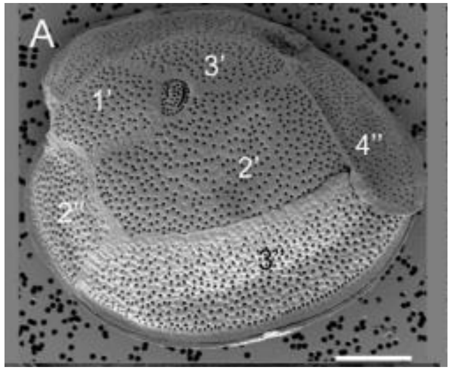
Toxin analyses (LC-MS) confirmed the presence of pectenotoxin-2 (PTX-2) in *D. tripos* from Spanish waters (Rodriguez et al. 2012)

Fabro et al. (2015) identified PTX-2, PTX-11 and PTX-2sa (but not OA) recurrently in association to *D. tripos* in the Argentine Sea and the first record of PTX11 and PTX-2sa for this area.

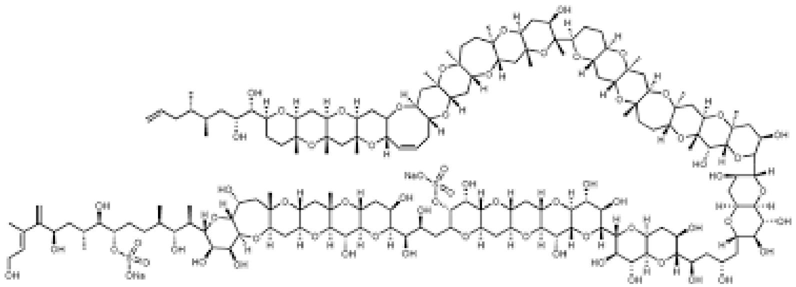
There have been no reports of DSP as a result of *D. tripos* in Australian waters to date.

Known Distribution in Australia	Common in Australian waters but rarely abundant; New South Wales coastal waters including Hawkesbury River (Ajani et al. 2001a, 2011, 2013b and 2016 submitted)
Known Seasonality	No detailed seasonal abundance data is available for this species in Australian waters however NSW wide phytoplankton monitoring data suggest presence in summer (unpublished data). Seen rarely in Hawkesbury River (Ajani et al. 2016 submitted)
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually present <500 cells L^{-1}; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Gambierdiscus carpenteri	Strains of *Gambierdiscus carpenteri* have been found to produce the toxin Maitotoxin (MTX) and its analog, MTX3. However, not every strain produces MTXs. Strains from Merimbula in NSW have not been found to produce MTX3 (Kohli et al. 2014).	*Gambierdiscus carpenteri* has forme a very dense bloom in Merimbula Lake Inlet, an estuary in southern NSW, in 2014, at water temperature of 16-17 degrees. It is generally epi-phytic or epi-benthic and inhabits macroalgae, seagrass, or the surrounding water column, in shallow habitats. It has also been sporadically reported from other sites in southern NSW. Strains from QLd have been found at Heron Island, an do appear to produce MTXs.



Kohli et al. 2014

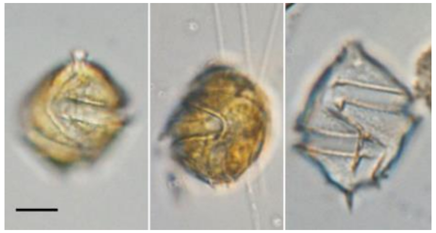


Known Distribution in Australia	NSW, at Merimbula, Wonboyn, Qld, Heron Island (Kohli et al. 2014)
Potential for human health risk	Maitotoxin has been found to accumulate in fish, but its toxic impacts on fish or shellfish are incompletely known.
Known Seasonality	Not known
Action Levels	**Not set, and incompletely known.**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☐ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity

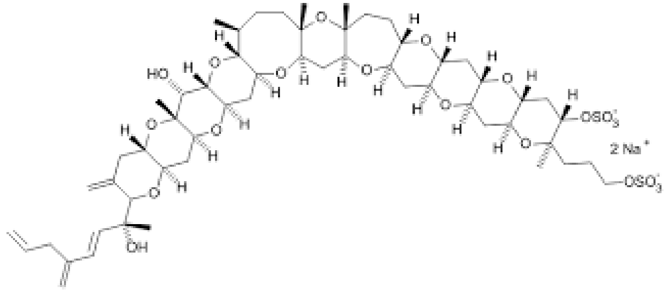
Species

Gonyaulax spinifera



Biotoxin

Gonyaulax spinifera from New Zealand was found to produce the toxin Yessotoxin and its analogues (Rhodes et al., 2006). Yessotoxin (YTX) is a disulfated polyether toxin.



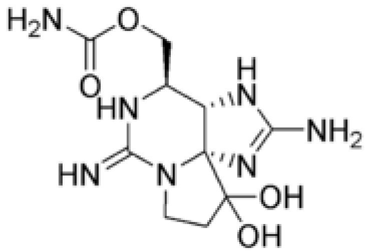
Mussel extracts contaminated by YTX cause high acute toxicity in mice, however, there are no reports of human intoxication caused by YTX (Toyofuku, 2006). Although YTXs may be of limited public health significance, at present the European legislation sets a limit of 1 mg YTX equiv/kg shellfish tissue.

Toxicity

Gonyaulax spinifera is not currently counted as part of regular biotoxin-focused phytoplankton monitoring in NSW, so its distribution in not known. *Gonyaulax* sp (not identified to species) have been identified in NSW estuarine waters (Ajani et al. 2001a, 2014a, b).

In the Adriatic Sea, blooms of *Gonyaulax spinifera* have led to the presence of YTXs in farmed shellfish, and this has been associated with lengthy closures of shellfish farms (Riccardi et al. 2009).

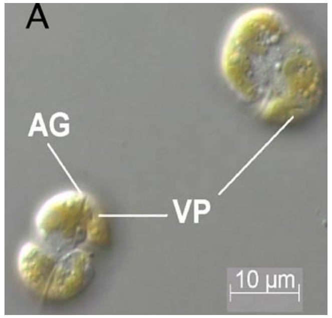
Known Distribution in Australia	Not known.
Potential for human health risk	Likely low
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Gymnodinium catenatum  	*Saxitoxin* (SXT) and its isomers, including the gonyautoxins and neosaxitoxin, are neurotoxins causing Paralytic Shellfish Poisoning (PST).  The main toxin components of *Gymnodinium catenatum* cultures from Tasmania, Australia are the C toxins, dcGTX3, GTX2,3 (Bolch et al. 1999, Murray et al. 2011, Negri et al. 2003).	In NSW, *Gymnodinium catenatum* has been found sporadically at several estuarine sites: Manning River, Brisbane Water, Hawkesbury, Jervis Bay, Tuross Lake, Nelson Lagoon and Merimbula Lake (Ajani et al. 2012). Generally, it has been present in low abundances, although it did exceed the PAL limit 4 times between 2005-2009 in NSW. Elsewhere in Australia, it has formed dense blooms, particularly in Tasmania, including in the Derwent and Huon Estuaries in 1985/86, which led to widespread closure of the local shellfish industry for several months (Hallegraeff and Sumner, 1986). Since then in Tasmania, *G. catenatum* has caused small localised annual blooms, and more recently, a large PST incident in Tasmanian waters in 2011, which led to PST uptake in mussels and abalone (McLeod et al, in review). Blooms in Tasmanian waters tend to occur during the period December to June, in water temperatures 12-18 °C (Hallegraeff and Fraga, 1998). It has also been found in southern Victoria and at Port Lincoln, South Australia. Worldwide, it forms dense blooms that result in PST in shellfish in Spain, Mexico, and other sites (Bolch and de Salas 2007, Band-Schmidt et al. 2010).

Known Distribution in Australia	Tasmania (Bolch et al. 1999, Bolch and de Salas 2007), South Australia (Bolch and de Salas 2007), NSW coastline (Ajani et al. 2013b, Bolch and de Salas 2007)
Potential for human health risk	Toxicity to humans is generally via consumption of seafood (shellfish, crabs, squid, octopus, fish).
Known Seasonality	In Tasmania, December-June, in NSW and Victoria, this is not yet known.
Action Levels	**Levels above 2000 cells L^{-1} can be associated with toxin uptake.**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species

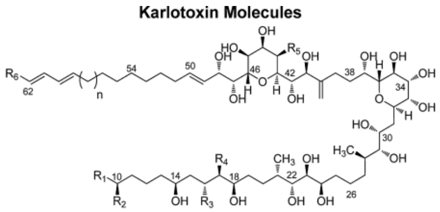
Karlodinium veneficum
(Syn: *Karlodinium micrum, Gymnodinium veneficum*)



Place et al. 2012

Biotoxin

The biotoxins involved in blooms of *Karlodinium veneficum* are called karlotoxins (KmTx), with several described congeners.



Place et al. 2012
KmTxs are lytic compounds that are highly active against blood cells, causing cell lysis. These compounds appear to impact the gills of fish and other marine life. Hypoxia also may result from high density blooms.

Toxicity

Karlodinium veneficum has been responsible for blooms linked to fish kills in NSW, in particular, a large scale fish kill in Jervis Bay in January 2011, which resulted in the deaths of >10,000 fish and rays in Hare Bay, in northern Jervis Bay (SM, unpublished data). It was also linked to a fish kill in Lake Illawarra in 2000 (Hallegraeff, 2015).

In WA, *K. veneficum* blooms regularly in the Swan River estuary (SRE) (1999, 2001, 2003, 2005, 2010, 2012) often causing fish kills. A bloom (10,000 cells ml^{-1}) occurred in the SRE in March-July 2005, and high levels of KmTx were detected (Adolf et al. 2015). The bloom was localized over a bottom layer of hypoxic water in a stratified water column, elevated phosphate and ammonium were present, while nitrate levels were low (Adolf et al. 2015), and salinity was 21-27 ppt. Blooms appear to develop under low flow conditions, and elevated flow rates appear to dissipate the blooms (Adolf et al. 2015).

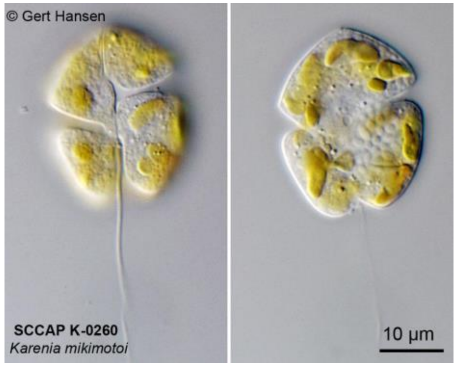
This species has also bloomed, causing fish kills, in the US (Chesapeake Bay, South Carolina estuaries), Spain, Norway, New Zealand, Singapore (Place et al. 2012).

Known Distribution in Australia	Not known, but likely to be widespread.
Potential for human health risk	May cause fish kills, and elevated pH that may cause skin irritations
Known Seasonality	Incompletely known. In NSW and WA, blooms have occurred previously in Summer- Autumn.
Action Levels	**Not set, and incompletely known. Fish kills in WA have occurred at levels of > 10^5 cells L^{-1}**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity

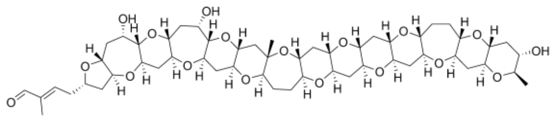
Species

Karenia mikimotoi
(Syn:: *Gymnodinium mikimotoi)*



Biotoxin

The biotoxins involved in blooms of *Karenia mikimotoi* are called Gymnocin A and B (Satake et al. 2002).



Gymnocin A

It appears that not all strains produce Gymnocin, and toxicity levels may vary. Toxicity may also be the result of toxic PUFA (Mooney et al. 2009). The deaths of fish and other marine life may also be caused by hypoxia when blooms are dense.
This species has caused mass mortalities, particularly of fish in fish farms, and gill damage is often apparent.

Toxicity

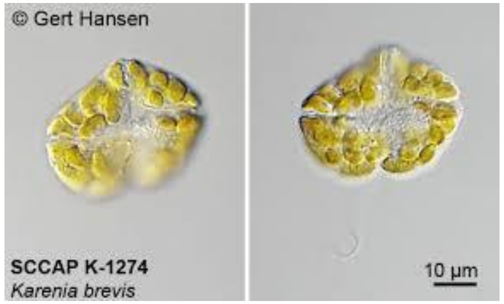
Karenia mikimotoi was likely responsible for a bloom linked to a fish kill in South Australia in 2014, as it was the most abundant species present (unpublished data). Fish kills linked to this species have not been reported elsewhere in Australia to date. Our genetic sequencing data indicates that this species is present at sites in NSW in low abundances (SM, unpublished data). In addition, it has occasionally been reported from NSW sites from the Hastings River to Wonboyn in southern NSW as part of regular phytoplankton monitoring (Ajani et al. 2013).

This species has also bloomed, causing fish kills, in the UK, Ireland, Norway, Japan, New Zealand, (Davidson et al. 2008). Modelling has linked *K. mikimotoi* blooms in European waters to sunlight driven phototaxis, rainfall mediated nutrient availability, and cell transport governed by wind direction/strength (Gentien, 1998).

Known Distribution in Australia	Port Lincoln, South Australia; NSW sites along coast, generally low abundances.
Potential for human health risk	May cause fish kills
Known Seasonality	Not known
Action Levels	**Not set. Levels of >10^3 cells L^{-1} have been associated with fish kills elsewhere (Davidson et al. 2008)**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☑ Fishing

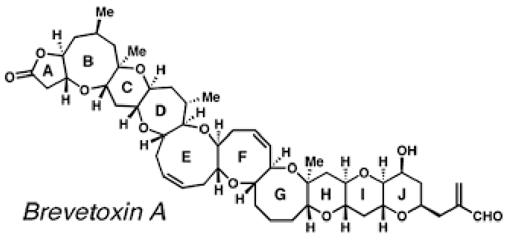
Species

Karenia brevis
(Syn: *Gymnodinium breve)*



Biotoxin

The biotoxins involved in blooms of *Karenia brevis* are multiple, including the brevetoxins, with 11 different congeners (Heil and Steidinger 2009). *K. brevis* produces an array of other polyketide compounds, including brevenal, and other related compounds. These toxins can accumulate in fish and shellfish, cause the mass deaths of fish and other marine life such as marine mammals, birds, and benthic species, and be aerosolised and caused breathing difficulties for beach goers.



Brevetoxins cause a syndrome called Neurotoxic Shellfish Poisoning when ingested.

Toxicity

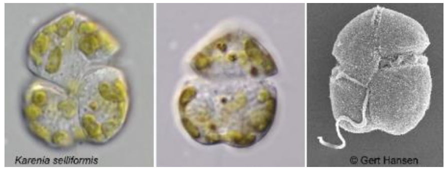
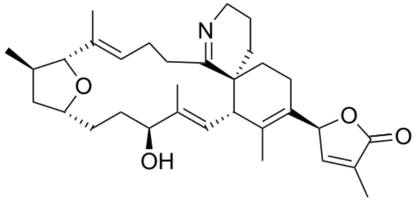
Karenia brevis has never been found in Australia, to our knowledge.

This species causes annual blooms in the US, in the Gulf of Mexico states such as Florida, Alabama, Texas (Heil and Steidinger, 2009), with an estimated loss of ~ $US 26 million per bloom.

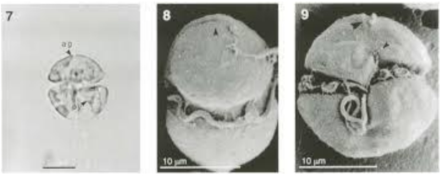
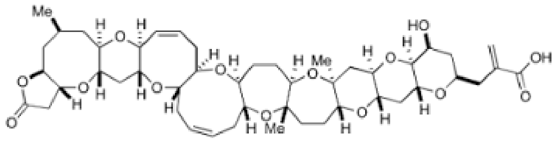
Blooms appear to be initiated offshore from the Gulf of Mexico, in oligotrophic marine rather than estuarine regions, over an extremely large latititudinal range (Heil and Steidinger, 2009). Blooms are then transported to inshore regions due to oceanographic processes.

K. brevis has also caused fish kills in Mexico (Heil and Steidinger, 2009).

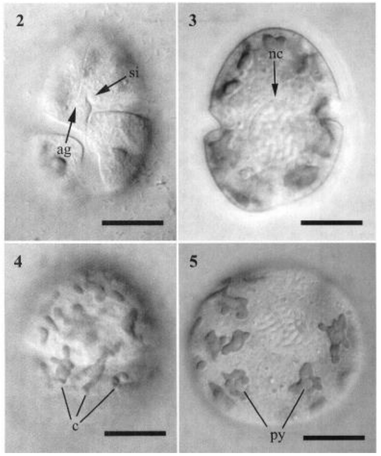
Known Distribution in Australia	Not known.
Potential for human health risk	May cause fish kills, toxins may accumulate in fish or shellfish, and inhaled toxins may cause respiratory problems in humans.
Known Seasonality	Not known to be present in Australia. In the US, no strong seasonality was found.
Action Levels	**In Florida, levels above 5 x 10^3 cells L^{-1} have led to toxin accumulation in shellfish and breathing irritation in humans.**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☑ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Karenia selliformis  Hansen et al.	The main toxin involved in blooms of *Karenia selliformis* has been found to be Gymnodimine and its isomers, (Seki et al. 1995; Miles et al. 2003),  *K. selliformis* has also been shown to produce other deleterious compounds such as hemolysins, known to lyse red blood cells (Tatters et al., 2010).	*Karenia selliformis* has been found in a sample from Stradbroke Island, Queensland, and from Tasmania (Hallegraeff 2015). It has not been associated with fish kills in Australia to date. It has been associated with widespread fish kills in New Zealand (Haywood et al. 2004) and in Tunisia (Feki et al. 2013), and has also been identified in samples from Florida, US. In addition, Gymnidimines from *K. selliformis* have been found to accumulate and persist in oyster and clam tissue for several years (Feki et al. 2013).

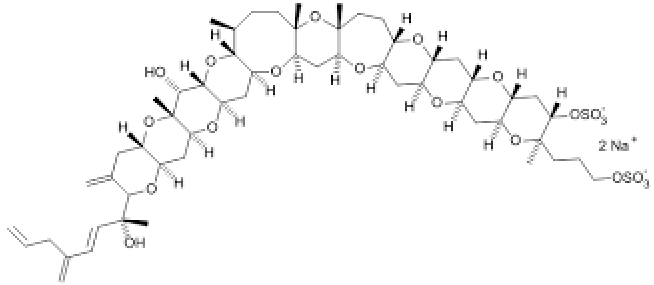
Known Distribution in Australia	Stradbroke Island, Queensland, and Tasmania
Potential for human health risk	May cause fish kills, and toxins accumulate in shellfish
Known Seasonality	Incompletely known.
Action Levels	**Not set, and incompletely known.**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Karenia brevisulcata (Syn: *Gymnodinium brevisulcatum)*  Chang 1999	The biotoxins involved in blooms of *Karenia brevisulcata* are called brevisulcatic acids (BSXs) and brevisulcenals (KBTs), both polycyclic ether toxins (Harwood et al. 2014).  brevisulcatic acid-5 (BSX-5) These toxins are highly potent. An LC/MS assay to detect them has been developed by the Cawthron Insitute in New Zealand (Harwood et al. 2014).	*Karenia brevisulcata* has never been found in Australia, to our knowledge. This species has bloomed, causing extensive fish kills, impacting almost all marine life in Wellington Harbour, New Zealand during the summer of 1998 (Chang 1999, Harwood et al. 2014). This included widespread and almost total mortality of bivalve molluscs and both pelagic and demersal fish species (Wear and Gardner, 2001). Approximately 90 people reported respiratory distress after being exposed to the bloom along affected coastlines in Wellington Harbour during the bloom (Chang 1999).

Known Distribution in Australia	Not known.
Potential for human health risk	May cause fish kills, and breathing difficulties.
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Karenia umbella  de Salas et al. 2004	The biotoxins involved in blooms of *Karenia umbella* are not known. Toxicity may be the result of toxic PUFA (Mooney et al. 2009). The deaths of fish and other marine life may also be caused by hypoxia when blooms are dense.	*Karenia umbella* was originally described from Australian (Tasmanian) waters (de Salas et al. 2004). It has also been found in South Australian (Port Lincoln) and Western Australian (Swan River) waters (Hallegraeff 2015). It was associated with the mortality of approximately 1000 caged rainbow trout (*Oncorhynchus mykiss*) at a salmonid fish farm in Murdunna, on the Tasman Peninsula south-eastern Tasmania, in December 1989 (de Salas et al. 2004). A further more serious mortality event involving 100,000 Atlantic salmon (*Salmo salar*) occurred at a neighbouring Tasmanian site in May 2003 (de Salas et al. 2004).

Known Distribution in Australia	Not known, but likely to be widespread.
Potential for human health risk	May cause fish kills, and elevated pH that may cause skin irritations
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

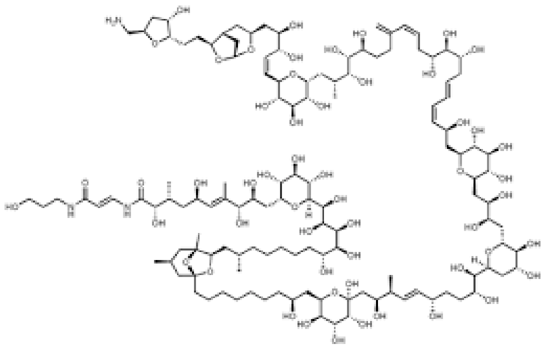
Species	Biotoxin	Toxicity
Lingulodinium polyedrum 	*Lingulodinium polyedrum* produces the toxin Yessotoxin and its analogues. Yessotoxin (YTX) is a disulfated polyether toxin that was first isolated from scallops in Japan (Murata et al. 1987).  Mussel extracts contaminated by YTX cause high acute toxicity in mice, however, there are no reports of human intoxication caused by YTX (Toyofuku, 2006). Although YTXs may be of limited public health significance, at present the European legislation sets a limit of 1 mg YTX equiv/kg shellfish tissue.	*Lingulodinium polyedrum* has been reported from the Hawkesbury during regular counts (S. Brett, unpublished) and bloomed in the Hawkesbury River (Calabash Bay) in Jan 2008 with two other potentially toxic species *Pseudo-nitzschia delicatissima* gp and Dinophysis caudata (Ajani et al. 2011). This species is not currently reported as part of regular biotoxin-focused shellfish program phytoplankton monitoring conducted in NSW estuaries. In the Adriatic Sea, the presence of YTXs in farmed shellfish has been associated with lengthy closures of shellfish farms (Riccardi et al. 2009).

Known Distribution in Australia	Occasionally reported from the Hawkesbury. Wider distribution in Australia not known.
Potential for human health risk	Likely low
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Ostreopsis siamensis	The biotoxins involved in blooms of *Ostreopsis siamensis* are called Palytoxins (Usami et al. 1995). Palytoxins can accumulate in seafood, cause human respiratory distress by way of inhalation, and lead to the deaths of marine life (Rhodes et al., 2002, Ciminiello et al., 2012).	*Ostreopsis siamensis* is an epi-benthic or epi-phytic species that has been found in shallow sub-tidal estuarine sites along the NSW coast (Verma et al. unpublished data). It has also been reported from Victoria in bloom abundances, and from Tasmania and Queensland. Human poisonings have not been reported from blooms of *O. siamensis* from Australia.

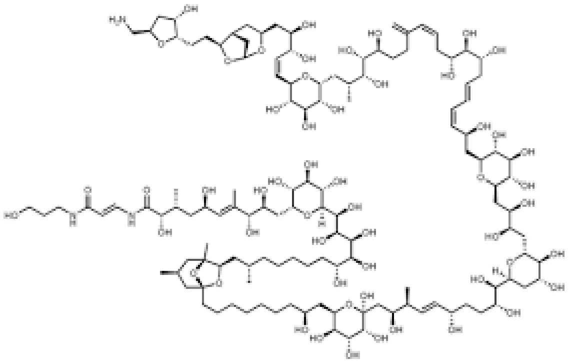
Verma et al. unpublished

O. siamensis strains from Australia have been found to produce Palytoxins and their analogs (Verma et al. unpublished data).

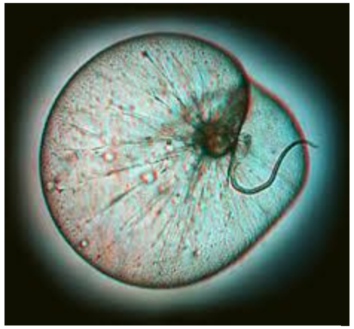


Blooms of *Ostreopsis siamensis* have been reported from the Mediterranean and New Zealand (Shears and Ross, 2009). During the bloom in NZ, *O. siamensis* abundance was strongly related to temporal and spatial variation in wave action, and were prevalent at sites protected from prevailing swells. Surveys of the health of sea urchins suggested strong negative effects on this ecologically important herbivore and urchin densities declined by 56–60% at bloom sites (Shears and Ross, 2009).

Known Distribution in Australia	Along NSW coastline, Qld, Victoria, Tasmania
Potential for human health risk	Toxins may accumulate in fish or shellfish, may cause the deaths of marine life, and inhaled toxins may cause respiratory problems in humans.
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☑ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Ostreopsis ovata  Verma et al. unpublished	The biotoxins involved in blooms of *Ostreopsis ovata* are called Palytoxins and their analogs (Usami et al. 1995). Palytoxins can accumulate in seafood, cause human respiratory distress by way of inhalation, and lead to the deaths of marine life (Rhodes et al., 2002, Ciminiello et al., 2012). *O. ovata* strains from Australia have been found to produce Palytoxins and their analogs (Verma et al. unpublished data). 	*Ostreopsis ovata* is an epi-benthic or epi-phytic species that is commonly found in warm shallow sites worldwide, and in Queensland in Australia. Human poisonings have not been reported from blooms of *O. ovata* from Australia. Blooms of *Ostreopsis ovata* have been reported from many countries, particularly in the Mediterranean region, (Italy, Spain, Greece and France) (Rhodes 2011). In France, *Ostreopsis. cf. ovata* has been associated with toxic events during 2006, off the coast of Marseille, and a specific monitoring has been designed and implemented since 2007. This showed that palytoxin accumulation (PLTX and ovatoxin-a) occurred in bivalve molluscs (mussels) and herbivorous echinoderms (sea urchins) (Amzil et al. 2012).

Known Distribution in Australia	Qld, Heron Island
Potential for human health risk	Toxins may accumulate in fish or shellfish, may cause the deaths of marine life, and inhaled toxins may cause respiratory problems in humans.
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☑ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Noctiluca scintillans  Wim van Egmond (microscopy-UK)	*Noctiluca scintillans* can produce elevated levels of ammonia in the water column, which can lead to skin irritations among swimmers. Fish also can be impacted by ammonia levels and low dissolved oxygen in regions of high density	*N. scintillans* is a very common bloom forming species in southern Australia (ie Murray and Suthers 1999) and worldwide. Due to the large size of the cells (up to 800 microns), the species is easily recognized. Along the NSW coast, *N. scintillans* is seasonally present during spring and summer, with typical values of 16 cells^{-1} (De la Cruz et al 2002). As this is a heterotophic species, blooms are stimulated by food availability, particularly diatoms, which can be stimulated by uplifted nutrients in coastal currents (De la Cruz et al 2002). While this species is often not abundant in comparison to other plankton, it can become concentrated in the surface layer by winds and tides, as cells are buoyant (Murray and Suthers 1999). If it becomes dense in a thin layer, it can discolour the water red. This species is often bioluminescent, and can cause spectacular displays at night. In 2012, it caused large scale closures of beaches in the Sydney region.

Known Distribution in Australia	Very common along the East Australian coastline, from Qld to Tasmania
Potential for human health risk	Skin irritations from Ammonia.
Known Seasonality	Year round. More common in Spring or late Summer (de la Cruz et al 2002, Murray et al 1999)
Action Levels	**Not set**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

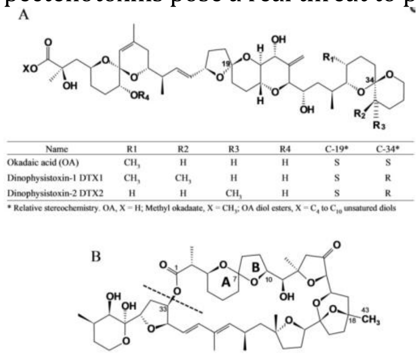
Species

Phalachroma mitra **(**formerly known as *Dinophysis***)**

Ajani 2014

Biotoxin

Two types of polyether toxins are produced by *Dinophysis* species **-** okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al. 2004).



Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

Reguera et al. 2014

Toxicity

No blooms or DSP events linked to *P. mitra* have been reported IOC-UNESCO).

DTX1 was detected using HPLC-FD analysis of picked *D. tripos* cells (Lee et al. 1989).

There have been no reports of DSP as a result of *P. mitra* in Australian waters to date.

Known Distribution in Australia	Rare in Australian coastal waters; New South Wales coastal waters (2013b & 2014a, b)
Known Seasonality	No detailed seasonal abundance data is available for this species in Australian waters however NSW wide phytoplankton monitoring data suggest presence in summer, winter and spring (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

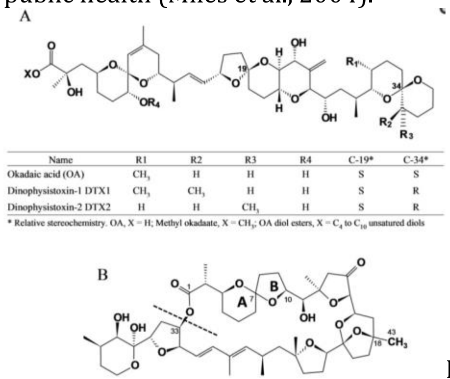
Species

Phalachroma rotundatum (formerly known as *Dinophysis****)***

Ajani 2014

Biotoxin

Two types of polyether toxins are produced by *Dinophysis* species - okadaates, or okadaic acid (OA) and its analogues the dinophysistoxins (DTX), and (ii) pectenotoxins (PTX). Okadaates are the only toxins with diarrheic effects, and there is at present controversy as to whether pectenotoxins pose a real threat to public health (Miles et al., 2004).



Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

Reguera et al. 2014

Toxicity

DTX1 was detected using HPLC-FD analysis of picked *D. tripos* cells (Lee et al. 1989).

Usually co-occurring with toxic species *D. acuminata, D. acuta, D. norvegica and D. caudata*, and may not be a toxin producer toxic itself. New results by Gonzalez-Gil et al. (2011) suggest that *P. rotundatum* does not produce toxins de novo, but acts as a vector from toxin-containing prey (eg. *Mesodinium*) to shellfish.

There have been no reports of DSP as a result of *P. rotundatum* in Australian waters to date.

Known Distribution in Australia	Common in Australian waters but rarely abundant; New South Wales coastal waters including Hawkesbury River (Ajani et al. 2001a, 2013b, 2014 a, b)
Known Seasonality	No detailed seasonal abundance data is available for this species in Australian waters however NSW wide phytoplankton monitoring data suggest presence all year round with maximum abundance in summer and spring (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates and marine gastropods. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species	Biotoxin	Toxicity
Prorocentrum mimimum *(Syn: Prorocentrum cordatum)*	*P. minimum* is observed to contain a neurotoxin which is yet to be characterised.	Certain strains of this species have been found to produce a water soluble neurotoxin (not yet characterised) which causes death to mice in high doses (Grzebyk et al. 1997). When fed on *P. minimum*, detrimental effects on scallops, oysters and clams have also been reported (Glibert et al. 2007). Poor larval development, tissue pathologies, systemic immune responses or no effect at all, were among the variable results from these feeding experiments (Wikfors 2005). Strain specific toxicity (Heil et al. 2005) or transient toxin expression (Wikfors 2005) are both possible explanations for this response variability. *P. minimum* has also been linked to fish, shellfish and zoobenthos mortalities, as well as being associated with human poisonings events in several countries throughout the world (Japan, France, Norway, Netherlands and USA) (Heil et al. 2005). Whilst toxin production has been unequivocally confirmed from the benthic forms of *Prorocentrum* (diarrhetic shellfish toxins including okadaic acid), there is no scientific consensus on the toxicity and human health effects associated with *P. minimum* thus far. This species bloomed in Berowra Creek in March 1995 and in March 2000 in Drummoyne Sydney Harbour. In March 2002, mortality (15-100%) of Sydney rock oysters was implicated as the causative agent in Wonboyn Lake, NSW (Ogburn et al. 2005).

(c) Station Biologique de Roscoff

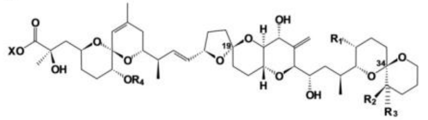
Known Distribution in Australia	Common in Australian waters and often seen in NSW coastal waters and all NSW estuaries, sometimes blooming (Ajani et al. 2001a, 2001b, 2011, 2013b, 2014a, b)
Known Seasonality	Maximum concentrations of this species in offshore waters have been observed in October (Ajani et al. 2001, 2014a, b). NSW wide phytoplankton monitoring data suggest presence all year round with maximum abundance in winter (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates, marine gastropods and possibly fish. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually present <500 cells L^{-1}; whilst counts up to 8.7x 10^5 cells L^{-1} have been reported from NSW estuaries (unpublished data)**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Prorocentrum lima	Several types of polyether toxins are produced by *Prorocentrum lima* – including okadaic acid (Murakami et al. 1982) and its analogues DTX-1 (Lee et al. 1989) and DTX-2 (Hu et al. 1993).	*Prorocentrum lima*, often referred to as the '*P. lima* complex', as there is still uncertainty surrounding its wide morphological and genetic variation, is a benthic/epibenthic species which can also be observed in the water column.

Ajani 2014

In addition, a prorocentrolide (Torigoe et al. 1988), a Fast Acting Toxin (FAT) (Tindall et al. 1984), and a new diol ester derivative of Dinophysistoxin-1 has been identified (Lee et al. 2015).

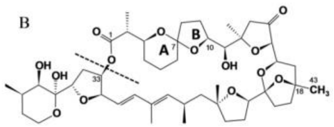
A



Name	R1	R2	R3	R4	C-19*	C-34*
Okadaic acid (OA)	CH_3	H	H	H	S	S
Dinophysistoxin-1 DTX1	CH_3	CH_3	H	H	S	R
Dinophysistoxin-2 DTX2	H	H	CH_3	H	S	R

* Relative stereochemistry. OA, X = H; Methyl okadaate, X = CH_3; OA diol esters, X = C_4 to C_{10} unsatured diols

B



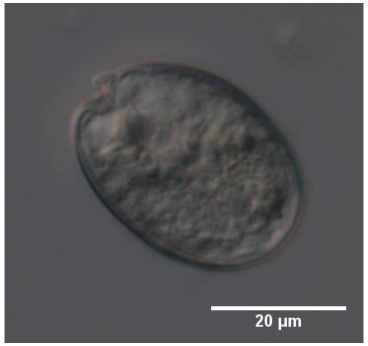
Densities are usually counted as dry weight of the macroalgal substrata and have been reported in the vicinity of 10^2 to 10^5 cells g-1 dry weight in temperate waters (Glibert et al. 2012 and references therein).

Its toxin production has been shown to be inversely related to nutrient limitation, increasing when nutrient ratios are about Redfield proportions (Glibert et al. 2012).

Known Distribution in Australia	Observed in majority of NSW estuaries and off shore at Port Hacking but never in high numbers (Ajani et al. 2011 & 2013b, 2014a, b)
Known Seasonality	NSW wide phytoplankton monitoring data suggest low abundance all year round with maximum abundance in spring (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates, marine gastropods and possibly fish. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☐ Fishing

Species

Prorocentrum rhathymum*



(c) Station Biologique de Roscoff

Biotoxin

P. rhathymum produces haemolytic toxins, not toxic to mice and a water soluble acetone precipitate which is toxic to mice. Some strains produce okadaic acid and its analogs (An et al. 2010), which lead to DSP, see above for information on okadaic acid.

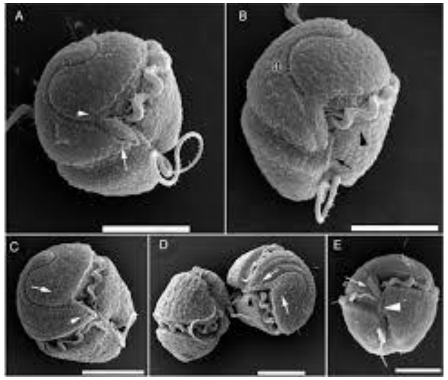
Toxicity

This species is mainly considered benthic and often confused with *P. mexicanum* but can form high-biomass planktonic blooms.

In response to an apparent association between oyster spat mortalities (up to 40%) and high *P. rhathymum* cell densities, Pearce et al. (2005) using brine shrimp, oyster bioassays and intraperitoneal mouse assays confirmed that fast acting toxins were preset in methanol but not aqueous extracts of *P. rhathymum.*

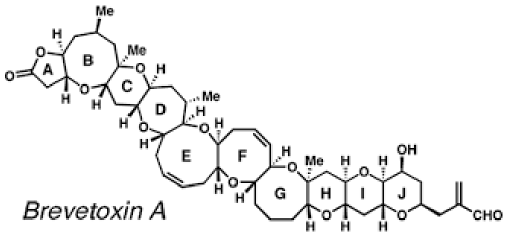
In March, 2007 this species bloomed in Lake Illawarra, but no effects were documented (Ajani et al. 2011)

Known Distribution in Australia	Observed in majority of NSW estuaries and off shore at Port Hacking but never in high cell densities (Ajani et al. 2013b, 2014a)
Known Seasonality	NSW wide phytoplankton monitoring data suggest presence all year round with maximum abundance in spring and early summer (unpublished data).
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates, marine gastropods and possibly fish. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**Usually rare; seafood toxicity testing 500 cells L^{-1}**
Impact	☐ Visual Amenity ☑ Fish kills ☑ Human Health
Warning	☐ Swimming ☑ Shellfish harvest ☑ Fishing

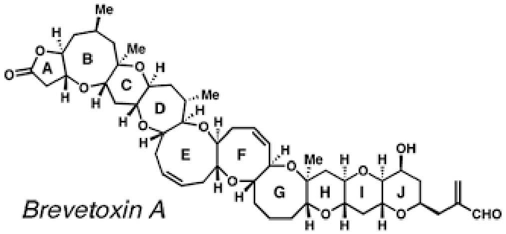
Species	Biotoxin	Toxicity
Takayama pulchella (Syn: *Gymnodinium pulchellum)*  de Salas et al., 2003	The biotoxins involved in blooms of *Takayama pulchella* are not known. Toxicity may be the result of toxic PUFA (Mooney et al. 2009). The deaths of fish and other marine life may also be caused by hypoxia when blooms are dense.	*Takayama pulchella* was described from Australian (Victorian) sites, as *Gymnodinium pulchellum* (Larsen 1994). It was thought to be linked to fish kills in the region, however, no molecular genetic data, cultures or toxicity information was available from the time to verify either the species identification or the toxins. *Takayama pulchella* has been widely reported along the NSW coastline from the Hastings River to Wonboyn (Ajani et al. 2013). *Takayama pulchella* was first recorded in Kagoshima Bay, Japan as an ichthyotoxic dinoflagellate (Onoue et al., 1985). *T. pulchella* has been linked to fish kills and the deaths of marine invertebrates in the Indian River, Florida, US and in China, in Xiamen Bay, in 1986 and in 2003, with a density up to 10^7 cells/L. (Larsen, 1994; Steidinger et al., 1998).

Known Distribution in Australia	Victoria, NSW, widespread from Hastings River to Wonboyn Lake.
Potential for human health risk	May cause fish kills.
Known Seasonality	Not known
Action Levels	**Not set**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

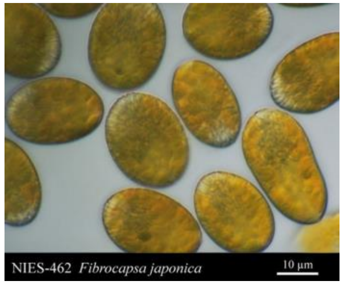
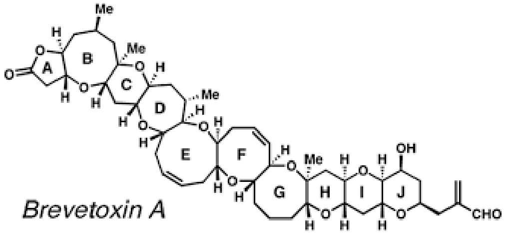
Chloromonads (Raphidophytes)

Species	Biotoxin	Toxicity
Chattonella marina  cultures.cawthron.org.nz	*C. marina* is an ichthyotoxic species which produces three neurotoxic components, CaTx-I, CaTx-II, and CaTx-III, which corresponded to Brevetoxin components PbTx-2, PbTx-3, and oxidized PbTx-2 (Khan et al. 1996). However, not all strains have been found to produce Brevetoxins, and fish kills may also occur through other means. 	*Chattonella marina* is a toxic raphidophyte responsible for red tides and fish kills. *C.* cf. *globosa* was the causative species for blooms in Canada Bay, Sydney Harbour from Nov 1996 to March 1997. When examining the ichthyotoxicity of *Chattonella marina* to damselfish (*Acanthochromis polycanthus*), it was found to contain a high concentration of the polyunsaturated fatty acid eicosapentaenoic acid (which has demonstrated toxic properties to marine organisms especially by damaging sensitive fish gills) which produces a synergistic toxic effect in the presence of ROS and free fatty acids (Marshall et al. 2003). In Australia, a mass mortality of caged blue fin tuna (*Thunnus maccoyii*) in April 1996 coincided with a bloom of *C. marina* at densities of 66,000 cell-l Marshall and Hallegraeff (1999). Brevetoxins were not definitively linked as the cause of the fish mortalities.

Known Distribution in Australia	Often seen in brackish waters, this species has been observed in South Australia, WA and Tasmania (Hallegraeff 2015)
Known Seasonality	NSW wide phytoplankton monitoring data suggest presence all year round with maximum abundance in autumn and spring (unpublished data).
Potential for human health risk	May cause fish kills, brevetoxins may accumulate in fish or shellfish, and inhaled brevetoxins may cause respiratory problems in humans.
Action Levels	**Relatively rare to observe but blooms of up to 66,000 cells L^{-1} have been observed (Marshall and Hallegraeff 1999).**
Impact	☑ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Heterosigma akashiwo  Ajani et al. 2001	Toxins produced by this species are neurotoxin substances HaTx-i, HaTx-iia, HaTx-nb and HaTx-iii which corresponded to Brevetoxin components PbTx-2, PbTx-9, PbTx-3 and oxidized PbTx-2 (Khan et al. 1996). However, not all strains have been found to produce Brevetoxins, and fish kills may also occur through other means.  Brevetoxin A	*Heterosigma akashiwo* is responsible for red tides throughout the world with blooms having up to several million cells per liter (O'Halloran et al. 2006). *H. akashiwo* has been found to be responsible for caged finfish kills in Japan, China, Korea, Norway, Canada, Chile, New Zealand and USA, resulting in billions of tonnes of lost fish worth millions of dollars (Khan et al., 1996). In British Columbia, *H. akashiwo* was responsible for the estimated loss of US 14 million dollars of farmed fish during 1986–1991 (Taylor and Haigh, 1993). *Heterosigma akashiwo* has recently bloomed in Aquaculture Prawn ponds in Queensland, causing extensive mortalities (SM, unpublished data).

Known Distribution in Australia	This species is found in NSW (Port Stephens and Berowra Creek), SA, WA, QLD and Tasmania (Ajani et al. 2001, Hallegraeff et al. 2015)
Known Seasonality	NSW wide phytoplankton monitoring data suggest presence all year round with maximum abundance winter (unpublished data).
Potential for human health risk	May cause fish kills, brevetoxins may accumulate in fish or shellfish, and inhaled brevetoxins may cause respiratory problems in humans.
Action Levels	**Usually present in low concentrations, but blooms have been identified in Queensland**
Impact	☑ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☑ Swimming ☐ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Fibrocapsa japonica  shigen.nig.ac.jp	This species is toxic to fish with possible mechanisms including the production of brevetoxins reactive oxygen species (ROS) haemolysins, haemoglutinating compounds, mucocyst threads or a combination of these vectors (Marshall et al., 2003 and references therein; Pezzolesi et al., 2010 and references therein). Not all strains appear to produce brevetoxins.  Brevetoxin A	When examining the toxic effect of *F. japonica* on the larvae of the common flatfish sole (*Solea solea*), ichthyotoxicity related primarily to the combination of endo- and exotoxins, with most probably not brevetoxins but, haemolytic PUFAs as the main endotoxins, and other haemolysins and ROS as the main exotoxins (Marshall et al. 2003). The combination of endo and exotoxins showed 100% mortality while endo- or exotoxins alone did not (Marshall et al. 2003).

Known Distribution in Australia	This species is found in Victoria, NSW and Tasmania (Ajani et al. 2013, Hallegraeff et al. 2015)
Known Seasonality	NSW wide phytoplankton monitoring data suggest this species is rare, with maximum abundance in autumn (unpublished data).
Potential for human health risk	May cause fish kills, brevetoxins may accumulate in fish or shellfish, and inhaled brevetoxins may cause respiratory problems in humans.
Action Levels	**Usually present in low concentrations, with maximum observed in NSW at 500 cells L-1**
Impact	☐ Visual Amenity ☑ Fish kills ☐ Human Health
Warning	☐ Swimming ☐ Shellfish harvest ☑ Fishing

Cyanobacteria (blue-green algae)

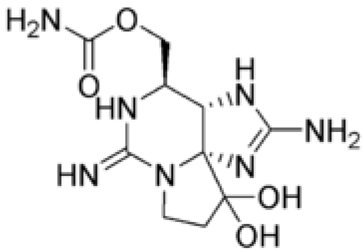
Species

Dolichospermum sigmoideum
(Syn: *Anabaena circinalis*)

oceandatacenter.ucsc.edu

Biotoxin

Saxitoxin (SXT) and its analogues are neurotoxins causing Paralytic Shellfish Poisoning (PST).



The main toxin components of *D. sigmoideum* cultures from Australia have been found to be C-toxins and gonyautoxins (Beltran and Neilan, 2000; Negri et al. 2003). Other species belonging to this genus are also toxic.

Toxicity

American and European isolates of *A. circinalis* produce only Anatoxin-a, while Australian isolates exclusively produce PSPs, potent sodium-channel blockers, which can lead to paralysis and death due to respiratory failure. Saxitoxin, the most poisonous of these, has an LD50 of 5 µg/kg of body weight, which means that a dose of only 0.39 mg could potentially kill the average adult male. (http://web.mst.edu/~microbio/BIO221_2010/A_circinalis.html). The reason for this geographical segregation of neurotoxin production is unknown, however, the PSP- and non-PSP-producing strains form two distinct 16S rRNA gene clusters (Beltran and Neilan, 2000).

Paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* has been found to accumulate to high concentrations (>80 mu g/100 g of mussel flesh) in the freshwater mussel *Alathyria condola* (Negri et al. 1995).

In Nov-Dec 1991 1000km of the Darling-Barwon river experienced an *A. circinalis* bloom with an estimated 10,000 deaths of livestock. This species also bloomed in Myall Lakes (NSW) throughout 1999 and early 2000.

Known Distribution in Australia	Freshwater species occasionally in brackish/marine waters.
Known Seasonality	Observation in estuaries suggests a late spring to autumn occurrence (mainly summer) or after heavy rainfall (Ajani pers. obs.)
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates, marine gastropods and fish, as well as through drinking water supplies. There is no evidence of toxin inhalation and no experiments to evaluate aerosol exposure to these toxins.
Action Levels	**General warning is to avoid any exposure to this organism including swimming or wading in areas where *Dolichospermum* may be blooming. Avoid direct contact with material washed onto the beach. Do not consume shellfish or fish from a bloom area. Keep pets and livestock away from bloom.**
Impact	✓ Visual Amenity ☐ Fish kills ✓ Human Health
Warning	✓ Swimming ✓ Shellfish harvest ✓ Fishing

Species

Moorea producens *(*syn. *Lyngbya majuscula* (common name ‘mermaid hair’, ‘stinging limu’ or ‘fire weed’)*.*

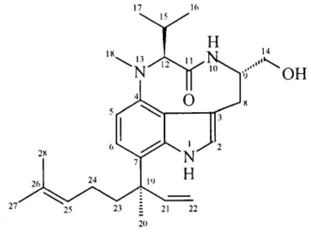
university.uog.edu

https://www.ehp.qld.gov.au/

Biotoxin

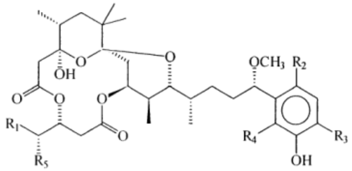
This species has been found to produce more than 70 biologically active compounds including lyngbyatoxin A (A) and debromoaplysiatoxin (B).

A.



LA

B.



Toxicity

L. majuscula is a benthic filamentous marine cyanobacterium that forms dense algal mats that float on the surface and drift with the current, or can wash up on beaches and cause amenity issues. It is generally a subtropical to tropical species and an important component of coral reef ecosystems. With ocean warming we may expect to see more blooms of this species in NSW.

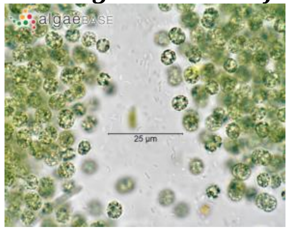
Blooms in Moreton Bay were first reported by fishermen who experienced dermatitis (“swimmers itch”) and asthma-like symptoms (Osborrne et al. 2008). The dermatitis condition is thought to be unique to Australia.

There is also the potential for the toxins produced by *Lyngbya majuscula* to affect ecological health (chemicals found in *L. majuscula* have been found to be feeding deterrents for many marine organisms). Toxins have also been detected in invertebrates (sea hares) that feed on *Lyngbya*. Fish generally avoid areas affected by *L. majuscula*.

Known Distribution in Australia	Predominantly subtropical and tropical waters of Australia with blooms reported in Moreton Bay, Queensland
Known Seasonality	Blooms can occur at any time of the year but are most commonly occurring between October and March when increased temperatures, sunlight and other environmental factors create favorable growth (https://www.ehp.qld.gov.au/)
Potential for human health risk	Toxicty to humans can be via consumption fish, turtles, invertebrates and seaweed. Skin effects and toxic aerosols observed.
Action Levels	**Avoid any exposure to this organism including swimming or wading in areas where *M. producens* may be blooming. Avoid direct contact with material washed onto the beach. Do not consume shellfish or fish from a bloom area. Keep pets and livestock away from bloom.**
Impact	✓ Visual Amenity ☐ Fish kills ✓ Human Health
Warning	✓ Swimming ✓ Shellfish harvest ✓ Fishing

Species

Microcystis spp. (M. weisenbergii, M. aeruginosa and M. flosaquae)

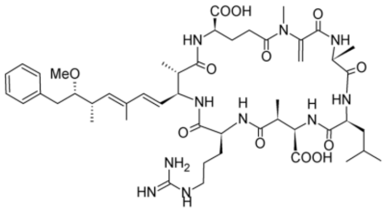


www.algaebase.org

http://www.ozcoasts.gov.au

Biotoxin

Toxins produced by these species are called microcystins (MCs) and are the most commonly occurring cyanotoxins posing a major threat to drinking and irrigation water. These toxins are potent inhibitors of the eukaryotic protein phosphatase families PP1 and PP2A leading to cell death (apoptosis). Studies have also shown that MCs play a role in liver toxicity and possibly even stimulate the growth of cancer cells following exposure.



Toxicity

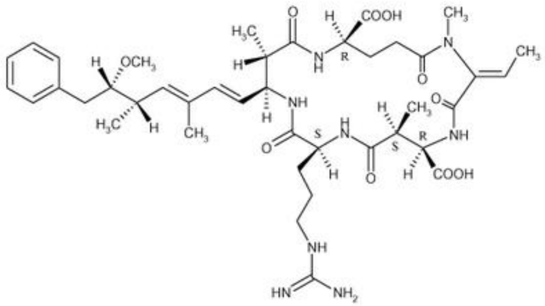
Species belonging to the genus *Microcystis* (*M. weisenbergii, M. aeruginosa and M. flosaquae* amongst others) are freshwater blue green algae that occasionally bloom in brackish/marine waters.

In early 2000, towards the end of an *Anabaena circinalis* bloom, *Microcystis aeruginosa* bloomed in Myall Lakes NSW with low levels of toxins detected in early February 2000 (Ajani et al. 2001b).

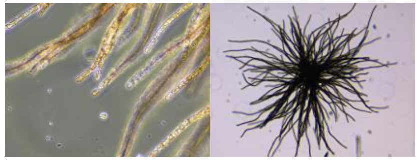
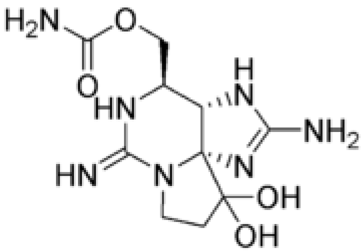
Four species of *Microcystis* have been observed in the Hawkesbury River (*M. aeruginosa, M. botrys, M. viride, M. weisenbergii*) although no species has bloomed in this area to date.

Microcystins are hepatotoxic and able to cause serious damage to the liver.

Known Distribution in Australia	Freshwater species occasionally in brackish/marine waters.
Known Seasonality	Observation in estuaries suggests a summer occurrence or after heavy rainfall (Ajani pers. obs.)
Potential for human health risk	Toxicty to humans via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates, marine gastropods and fish, as well as through drinking water supplies. There is evidence of aerosol exposure to these toxins (Murby and Hanley 2015 and references therein)
Action Levels	**Metropolitan South Coast Regional Algal Co-ordinating Committee have alert levels – green > 500 to < 5,000 cells/mL, amber ≥ 5,000 to < 50,000 cells/mL and red ≥ 50,000 cells/mL. General warning is to avoid any exposure to this organism including swimming or wading in areas where *Microcystis* may be blooming. Avoid direct contact with material washed onto the beach. Do not consume shellfish or fish from a bloom area. Keep pets and livestock away from bloom.**
Impact	☑ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☑ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Nodularia spumigena 	*N. spumigena* produces nodularin-R, a potent hepatotoxin that may cause serious damage to the liver of humans and other animals (Carmichael et al. 1988, Sivonen et al. 1989). 	A blue green filamentous algae, this species blooms in estuarine systems and coastal embayments of Australia. Blooms have been reported to cause deaths of pets, livestock and wild animals (including fish and birds) that drink from ponds, dams, lakes and reservoirs. Fish and crustacea are also reported to avoid estuaries affect by *Nodularia* blooms (Hallegraeff, 1991: 94). Details of toxic effects are detailed in Hallegraeff 2015 and include toxin accumulation in mussels, prawns and fish. Fish and crabs avoid the bloom and commercial catches can be affected. Symptoms in humans have been reported such as stomach complaints, headaches, eczema and inflammation of the eyes (Hallegraeff 2015 and references therein). Work on Australian strains by Blackburn et al. 1996 suggest that mature blooms are more toxic than developing ones because of their greater biomass and higher toxin content.

Known Distribution in Australia	Reported to form dense blooms in Vic, Tas, WA and SA (Hallegraeff 2015 and references therein).
Known Seasonality	Bloom ususally reported in the warmer summer months
Potential for human health risk	Toxicty to humans only via consumption of bivalve molluscs (mussels, scallops, oysters and clams) echinoderms, tunicates, marine gastropods and fish, as well as through drinking water supplies. Skin effects and evidence of aerosol exposure to these toxins (Murby and Hanley 2015 and references therein).
Action Levels	**Avoid any exposure to this organism including swimming or wading in areas where *Noduclaria* may be blooming. Avoid direct contact with material washed onto the beach. Do not consume shellfish or fish from a bloom area. Keep pets and livestock away from bloom.**
Impact	☑ Visual Amenity ☐ Fish kills ☑ Human Health
Warning	☑ Swimming ☑ Shellfish harvest ☑ Fishing

Species	Biotoxin	Toxicity
Trichodesmium erythraeum (common name 'sea sawdust'; 'sea scum')   Ajani (pers. comm.)	Experiments feeding homogenized *Trichodesmium* to copepods suggest that this blue green algae contains certain type(s) of intracellular biotoxins (Guo & Tester 1994). Although not identified to species level, saxitoxin concentrations (STX-eq) ranging from 0.45 to 3.9 mg L-1 were reported from *Trichodesmium* blooms in southwestern South Atlantic Ocean, with GTX-4 being the main variant of this neurotoxin in the blooms (Detoni et al. 2016). 	The cyanobacterium *Trichodesmium erythraeum* is also a common red tide‟ organism in NSW coastal waters. This tropical/subtropical species produces episodic blooms that were historically reported as 'sea sawdust' during Captain Cook‟s voyage through the Coral Sea (Cribb 1969). The filaments of this alga are united into small bundles that are just visible to the naked eye (~1mm). Blooms are most commonly seen in northern NSW waters in spring, summer and early autumn when the East Australian Current (EAC) transports these algal masses into NSW from Queensland waters. Blooms of this species can appear yellow-grey in their early stages, while later they become a reddish brown. Whilst they traditionally are considered non-toxic blooms in Australia (Hallegraeff 2015 and references therein), they can form dense blooms that may cause anoxic conditions and produce a "fishy" odour. Blooms can be reported as resembling spilt paint or oil slicks in the ocean. The taxonomy of *Trichodesmium* in Australia is unclear, with both bundle forming colonies (*T. erythraeum*?) and more recently radiating colonies (*T. thiebautii*?) seen at Port Hacking long term coastal station (Ajani pers. obs.). With this in mind *T. thiebautii* has been observed to contain a neurotoxin (Codd 1994) and cause respiratory difficulties ('*Trichodesmium* fever' Sato et al. 1963).

Known Distribution in Australia	Observed in temperate, subtropical, tropical waters around Australia.
Known Seasonality	*Trichodesmium* occurs in NSW waters nearly all year round, but especially in summer and autumn (Ajani et al. 2001, 2011, 2014 a & b).
Potential for human health risk	*Trichodesmium* is not considered toxic in Australian waters (Hallegraeff 2015 and references therein).
Action Levels	**Avoid any exposure to this organism including swimming or wading in areas where *Trichodesmium* may be blooming. Avoid direct contact with material washed onto the beach. As a precaution, do not consume shellfish or fish from a bloom area.**
Impact	✓ Visual Amenity ☐ Fish kills ? Human Health
Warning	✓ Swimming ? Shellfish harvest ? Fishing

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Appendix 1. New South Wales Food Authority's Marine Biotoxin Management Plan (MBMP, 2015) Phytoplankton Action Levels (as Appendix 6 in the MBMP 2015 plan)

Phytoplankton species	Toxin	Trigger flesh sampling# (cells per litre)	Alert level – Close harvest area pending flesh testing results	Issue public health warning (cells per litre)
Alexandrium minutum#	PSP	200	500	5000
Alexandrium ostenfeldii#	PSP	200	500	5000
Alexandrium catenella#	PSP	200	500	5000
Alexandrium tamarense#	PSP	200	500	5000
Alexandrium spp#.	PSP (?)			
Gymnodinium catenatum	PSP	1000 mussels 2000 other shellfish	5000	5000
Pseudonitzschia (P.multiseries & P.australis)*	ASP	50,000	500,000	N/A
Pseudonitzschia delicatissima group - historically non-toxic in Australia	ASP (?)	500,000		N/A
Karenia cf brevis	NSP	1000		5000
Dinophysis acuminata	DSP	1000		N/A
Dinophysis acuta	DSP	500		N/A
Dinophysis caudata	DSP	500		N/A
Dinophysis fortii	DSP	500		N/A
Dinophysis hastata	DSP	500		N/A
Dinophysis mitra	DSP	500		N/A
Dinophysis rotundata	DSP	500		N/A
Dinophysis tripos	DSP	500		N/A
Total Dinophysis spp.	DSP	500		N/A
Prorocentrum lima	DSP	500		N/A

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

The cell levels within each toxin group are cumulative, eg 600 cells/l of both *D. acuta* and *D. fortii* would mean a total count of 1200 cells/l, exceeding the critical level to initiate flesh testing.

Alexandrium species may be difficult to identify when numbers are low. If any doubt exists, they should be treated as potentially toxic.

* Species within the Pseudo-nitzchia groups are difficult to identify. The toxic species of most concern in each group are listed for those laboratories that have capacity to identify these algae to species level. Otherwise all algae within these groups should be considered potentially toxic.

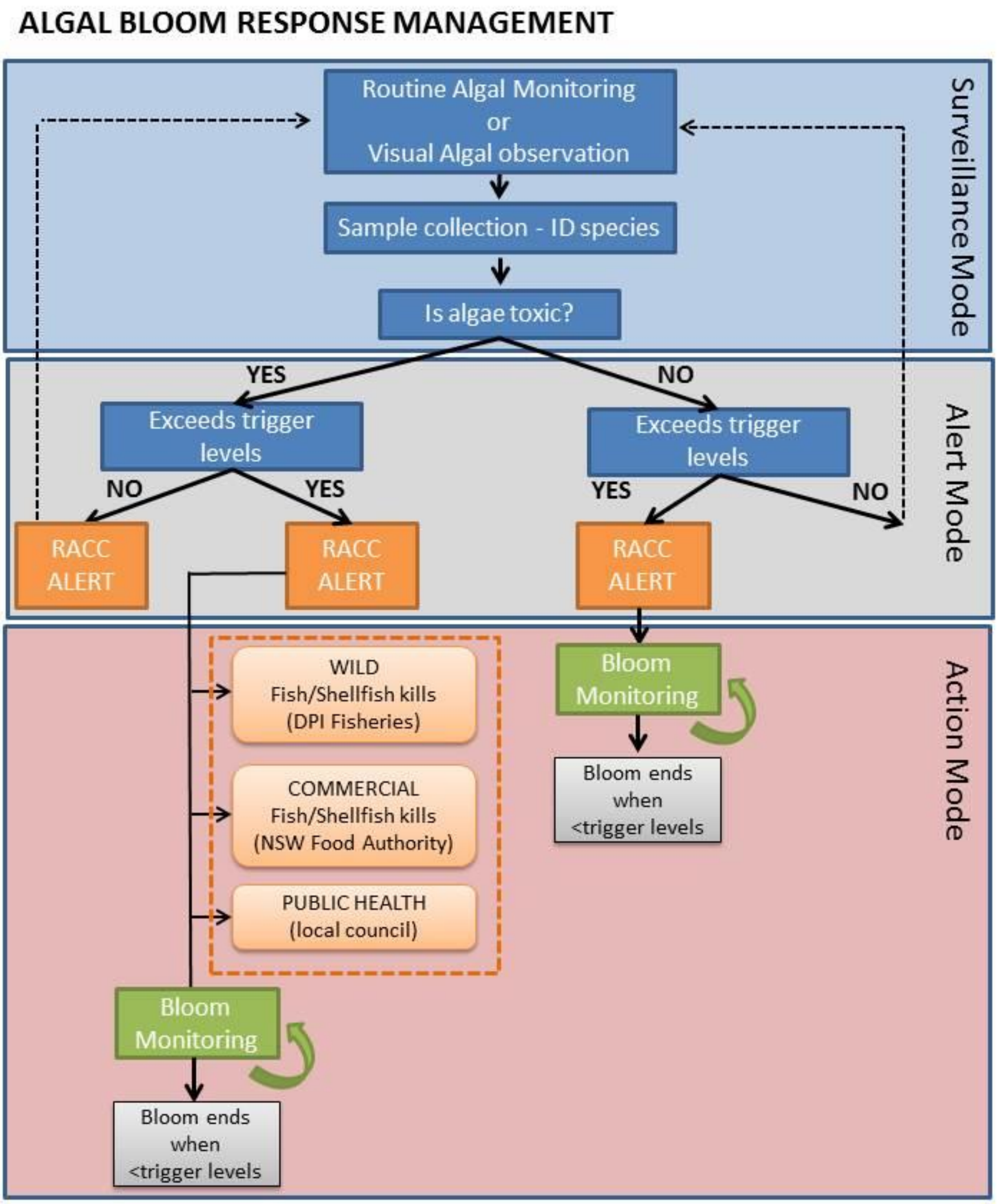
Appendix 2. National Health and Medical Research Council Guidelines (NHMRC 2008) for toxic syndromes and causative microalgal species.

Table 7.1 Toxic syndromes associated with marine algal toxins affecting humans

Syndrome	Causative organisms	Primary vector	Toxin	Pharmacologic target	Reference
Paralytic shellfish poisoning	*Alexandrium* sp *Gymnodinium* sp *Pyrodinium* sp *Karenia* sp	Shellfish	Saxitoxins	Binds to site 1 on the voltage-dependent sodium channel, affecting the nervous system.	Andrinolo *et al* 2002 Azanza *et al* 2001 Yoshida *et al* 2000 Compagnon *et al* 1998
Neurotoxic shellfish poisoning	*Karenia* sp *Gymnodinium* sp	Shellfish, aerosol	Brevetoxins	Binds to site 5 on the voltage-dependent sodium channel, affecting the nervous system. Aerosol linked to respiratory problems.	Chang *et al* 2001 Poli *et al* 1986 Trainer *et al* 1994 Dechraoui *et al* 1999
Ciguatera fish poisoning	*Gambierdiscus* sp	Reef fish	Ciguatoxins	Binds to site 5 on the voltage-dependent sodium channel, affecting the nervous system.	Lehane 2000, Holmes 1998, Lewis 2001 Dechraoui *et al* 1999 Hokama and Yoshikawa-Ebesu 2001
Amnesic shellfish poisoning	*Pseudo-nitzschia* sp	Shellfish	Domoic acid	Binds to subtypes of the glutamate receptor, resulting in both gastrointestinal and neurologic effects.	Mos 2001 Amzil *et al* 2001 Bates 2000
Diarrhoetic shellfish poisoning	*Dinophysis* spp *Prorocentrum* sp	Shellfish	Dinophysistoxins Okadaic acid	Inhibit ser/thr protein phosphatases 1, 2A and at high concentrations 2B, resulting in diarrhoea, impaired balance and loss of fluids. Tumour promoters	Ten-Hage *et al* 2000 Dahl and Johannessen 2001 Bravo *et al* 2001 Burgess and Shaw 2001 Marasigan *et al* 2001
Hepatotoxicity	*Nodularia spumigena*	Water	Nodularin	Inhibition of protein phosphatases 1 and 2A, breakdown of hepatic structure liver function with liver failure at high levels. Long-term exposure could promote liver cancer.	Kuiper-Goodman *et al* 1999
Estuary syndrome	*Pfiesteria* sp	Water	Unknown	Unknown target. Causes memory loss, confusion and respiratory, skin and gastrointestinal problems.	Morris 2001 Samet *et al* 2001
Swimmers itch — skin irritation	*Lyngbya majuscula*[a] *Oscillatoria nigroviridis*[a] *Schizothrix calcicola*[a]	Water	Debromoaplysiatoxin Lyngbyatoxin A	Protein kinase C. Causes dermatitis. Unknown target Unknown target	Hashimoto *et al* 1976 Osborne *et al* 2001 Mynderse *et al* 1977
Skin irritation	*Trichodesmium spp* *Heterosigma akashiwo*	Water	Unknown	Unknown target causing dermatitis.	WHO 2003

a Lyngbya majuscula is known to produce debromoaplysiatoxin and lyngbyatoxin A, and Oscillatoria nigroviridis and Schizothrix calcicola are known to produce debromoaplysiatoxin.

Appendix 3. NSW Algal bloom response management flow chart. This flow chart highlights the process in identifying, confirming and responding to an algal bloom in NSW waterways (developed by Hornsby Shire Council)



Appendix 4 Regional Algal Coordinating Committee (RACC) alert process once an algal bloom has been identified and quantified (developed by Hornsby Shire Council)

RACC ALERT PROCESS:

1. Notify RACC – request advice + disseminate among stakeholders (DPI Fisheries, NSW Food Authority, NPWS, NSW Health, local community organisations, local council, etc)
2. Report in EPA Pollution Line if relevant 131 555 (A/H)
3. Relevant management authority to investigate issue: algal bloom / fish kill/public illness
4. Consider erect warning signs based on relevant management authority protocol:
   i.e. include algal species identified + management strategy:
   - ☑ close recreational fishing area (DPI Fisheries)
   - ☑ close commercial harvest areas (NSW Food Authority)
   - ☑ close recreational swimming site (primary contact, local council)
   - ☑ close recreational boating site (secondary contact, local council)
5. Update algal hotline 1800 999 457
6. Include information in RACC weekly report
7. Media release and social media announcement
8. Lifting alerts: low counts in two consecutive samples taken within 7 days or once prevailing environmental conditions have changed (i.e. rainfall, run-off)
9. Update progress in RACC weekly report