PHYTOPLANKTON AND BIOTOXINS IN NSW SHELLFISH AQUACULTURE AREAS

RISK ASSESSMENT

NSW GOVERNMENT
Department of Primary Industries
Food Authority

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JUNE 17

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Executive summary

Phytoplankton are microscopic organisms that are the primary producers at the base of the food chain in almost all aquatic ecosystems. Some phytoplankton produce toxic compounds that can accumulate in filter-feeding bivalve shellfish and can be harmful to humans, if consumed. In each shellfish aquaculture producing estuary in NSW, local shellfish programs collect samples for phytoplankton and biotoxin analysis to monitor for potential risks from toxic phytoplankton. During the open harvest status, fortnightly phytoplankton sampling and monthly biotoxin sampling is conducted in accordance with the NSW Marine Biotoxin Management Plan and carried out in National Association of Testing Authorities (NATA), Australia accredited laboratories. Water samples are scrutinised for the presence of potentially harmful species and shellfish flesh is tested for the three main algal toxin groups found in NSW coastal waters: amnesic shellfish toxin, paralytic shellfish toxins and diarrhetic shellfish toxins.

The current NSW Shellfish Program began in 2003, following the commencement of formal classification on the sanitary status of NSW shellfish aquaculture areas. Phytoplankton and biotoxin monitoring commenced in 2004, with regular routine sample data collected from 2005. This risk assessment reviews the presence of potentially harmful species and positive biotoxin results during the life of the current program.

This report assessed 22,203 flesh tests for biotoxin analysis, along with 15,082 water samples for phytoplankton enumeration between January 2004 and December 2016. During this period, regulatory limits for algal biotoxins were exceeded during two separate bloom events. An anomalous and immense bloom of the amnesic shellfish toxin-producing *Pseudo-nitzschia cuspidata* occurred in Wagonga Inlet during 2010. Thirteen biotoxin samples from this event exceeded regulatory limits for amnesic shellfish toxin (Domoic acid) in Sydney rock oysters. During 2016, regulatory limits for paralytic shellfish toxins were exceeded in nine blue mussel samples collected from Twofold Bay during a bloom of *Alexandrium* spp. on the NSW south coast. No illnesses in seafood consumers were reported from either event.

In NSW shellfish aquaculture areas, significant phytoplankton blooms have been infrequent. The majority of harvest area closures have been due to rainfall and/or salinity exceeding the management plan limits used as indicators of microbial and viral water quality. However, increasing demand on coastal resources from an increasing population and the potential for spatial and temporal distributions of harmful phytoplankton to be altered dramatically by a changing climate are key future challenges. Since 2012, quantitative methods of biotoxin analysis are available where a value for toxin concentration in shellfish tissue (if present) is reported, rather than a positive or negative result only. With regards to routine biotoxin risk assessment this enables a better-informed approach for management of shellfish aquaculture programs, including a faster return to harvest on some occasions, and potential public health risks.

Introduction

Background

Farm gate value of the New South Wales (NSW) shellfish industry is in excess of $AUD30 million annually (Trenaman et al., 2015). The native *Saccostrea glomerata* is the principle species cultured, followed by *Crassostrea gigas* (diploid and triploid), *Ostrea angasi* and *Mytilus edulis* in selected harvest areas. Since 2005, management of NSW shellfish aquaculture areas has included routine phytoplankton and biotoxin monitoring. Currently there are 74 classified shellfish harvest areas across 28 embayments and estuaries (Figure 1).

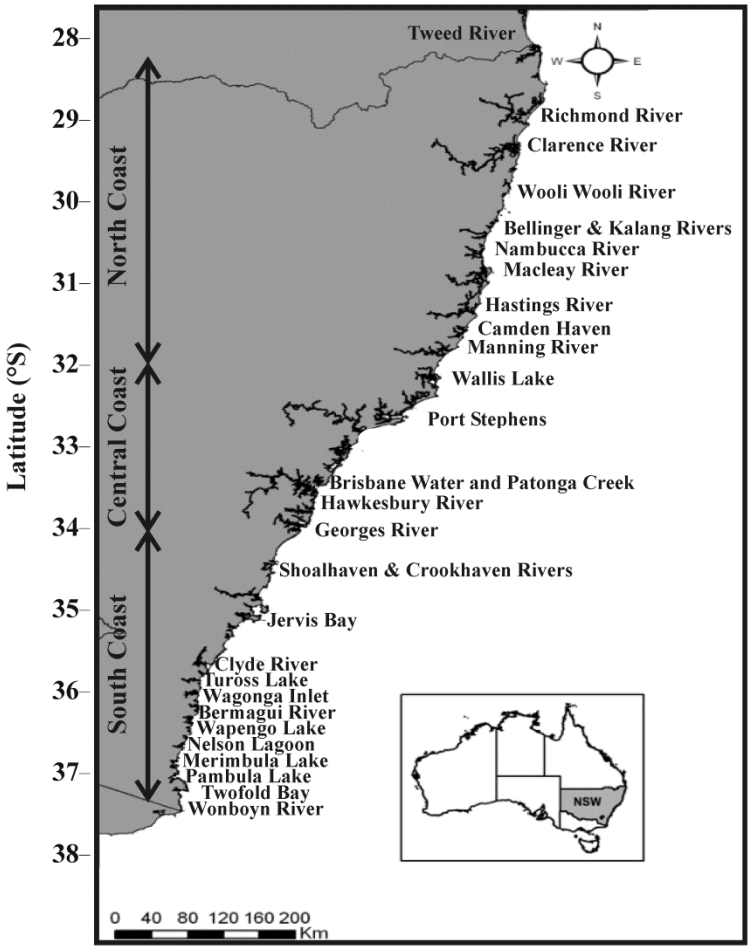

Figure 1 NSW estuaries and embayments that host currently classified shellfish aquaculture areas. Note: Jervis Bay does not currently host classified aquaculture areas but the area is expected to resume operations during 2017.

Risk assessment process

This risk assessment was based on the principles of the Codex Alimentarius Commission definition as *'a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization'* (WHO/FAO, 2015).

Hazard identification

> *'The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.'*

Phytoplankton are microscopic organisms that are the primary producers at the base of the food chain in almost all aquatic ecosystems. Some phytoplankton produce toxic compounds that can accumulate in filter-feeding bivalve shellfish and can be harmful to humans, if consumed. Some phytoplankton blooms can appear as water discolouration, including red, green, yellow, brownish or an oily or milky appearance. Scum may form on the water surface. The water may have a musty, earthy or pungent smell. Other blooms are not visible but are highly toxic even at low levels.

In Australia, the level of biotoxins in shellfish is regulated in *Standard 1.4.1 clause 3* of the Australian New Zealand Food Standards Code (the Code). The limits are similar to those regulated by the EU and in the USA (Table 1, EC-853 (2004), NSSP/US FDA (2013)). The three main algal toxin groups found in NSW coastal waters are amnesic shellfish toxin (AST), paralytic shellfish toxins (PSTs) and diarrhetic shellfish toxins (DSTs). Neurotoxic shellfish toxins (NSTs) and azaspiracid shellfish toxins (AZTs) have not previously been detected in NSW however, phytoplankton samples are routinely screened for all potentially harmful toxin producing species and the current biotoxin laboratory has the capacity to test for these additional toxin groups, if necessary. While a regulatory limit for AZTs is not currently defined in the Code, international standards (Table 1) would be applied to any positive AZT report.

Between 1999 and 2001, the Cawthron Institute in New Zealand was the lead agency in a collaboration between Australian and New Zealand regulators and scientists tasked with providing a national marine biotoxin strategy. The report is commonly referred to as the Cawthron Report (Todd et al., 2001) and was originally used to determine a list of potentially toxin-producing phytoplankton in Australian waters. This list is the basis of the routine monitoring assessment for potentially harmful species in NSW shellfish aquaculture areas. The current NSW Marine Biotoxin Management Plan (MBMP) (NSW FA, 2014) lists potentially harmful phytoplankton which are known to produce toxins in Australia or internationally and assigns 'phytoplankton action limits' (PAL) in seawater samples collected from NSW shellfish aquaculture areas that trigger further sampling or harvest area closure in the case of a phytoplankton bloom (Tables 2-5). The cell concentrations for each potentially toxic species are cumulative within a sample. There is a high level of technical expertise required to correctly identify toxin-producing phytoplankton. If any doubt exists, they are considered as potentially toxic. Taxonomic name changes since the Cawthron report have been incorporated into the NSW MBMP by the NSW Food Authority in consultation with the phytoplankton laboratory and other national and international phytoplankton experts.

Table 1 Regulatory limits for biotoxins in the EU, USA and Australia.

Toxin	EU limit Chapter V (2) (c) and (e) of Section VII of Annex III to Regulation (EC) No 853/2004	USA limit USFDA / NSSP	Australian limit Standard 1.4.1, clause 3 of the Food Standards Code.	Potential causative phytoplankton in NSW coastal waters
Amnesic shellfish toxin (AST) (Domoic acid (DA) equivalent)	20 mg/Kg	20 mg/Kg	20 mg/Kg	*Pseudo-nitzschia* spp.
Diarrhetic shellfish toxins (DSTs) (Okadaic acid (OA) equivalent)	0.16 mg/Kg	0.16 mg/Kg	0.2 mg/Kg	*Dinophysis* spp. *Prorocentrum lima*
Paralytic shellfish toxins (PSTs) (Saxitoxin (STX) equivalent)	0.8 mg/Kg	0.8 mg/Kg	0.8 mg/Kg	*Alexandrium* spp. *Gymnodinium catenatum*
Neurotoxic shellfish toxins (NSTs)^		0.8 ppm/ 200 Mouse Units/Kg	200 Mouse Units/Kg	*Karenia* spp. *Chatonella* spp. *Fribrocapsa japoinica* *Heterosigma akashiwo*
Azaspiracid shellfish toxins (AZTs)*	0.16 mg/Kg	0.16 mg/Kg		*Azadinium* spp.

^NSTs have not been previously reported in NSW. Routine phytoplankton samples are screened for potential NST-producing species. *AZTs have not been previously reported in NSW, the screen method for DSTs and AST used by Advanced Analytical Laboratory can detect at least three AZT analogues.

Table 2 Phytoplankton action limits for potential PST producing species in seawater samples collected from NSW shellfish aquaculture areas (NSW FA, 2014).

Phytoplankton species	Toxin	Trigger flesh sampling (cells/L)	Alert level – close harvest area pending flesh testing results	Issue public health warning (cells/L)
*Alexandrium minutum**	PST	200	500	5,000
*Alexandrium ostenfeldii**	PST	200	500	5,000
*Alexandrium catenella**	PST	200	500	5,000
*Alexandrium tamarense**	PST	200	500	5,000
Alexandrium spp.*	PST			
Gymnodinium catenatum	PST	1000 mussels 2000 other shellfish	5,000	5,000

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

Table 3 Phytoplankton action limits for potential AST producing species in seawater samples collected from NSW shellfish aquaculture areas (NSW FA, 2014).

Phytoplankton species	Toxin	Trigger flesh sampling (cells/L)	Alert level – close harvest area pending flesh testing results	Issue public health warning (cells/L)
Pseudo-nitzschia (*P. multiseries* and *P. australis*)*	AST	50,000	500,000	N/A
Pseudo-nitzschia delicatissima group – historically non-toxic in Australia	AST (?)	500,000		N/A

*Species within the *Pseudo-nitzschia* 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.

Table 4 Phytoplankton action limits for potential DST producing species in seawater samples collected from NSW shellfish aquaculture areas (NSW FA, 2014).

Phytoplankton species	Toxin	Trigger flesh sampling (cells/L)	Alert level – close harvest area pending flesh testing results	Issue public health warning (cells/L)
Dinophysis acuminata	DST	1,000		N/A
Dinophysis acuta	DST	500		N/A
Dinophysis caudata	DST	500		N/A
Dinophysis fortii	DST	500		N/A
Dinophysis hastata	DST	500		N/A
Dinophysis mitra	DST	500		N/A
Dinophysis rotundata	DST	500		N/A
Dinophysis tripos	DST	500		N/A
Total *Dinophysis* spp.	DST	500		N/A
Prorocentrum lima	DST	500		N/A

Table 5 Phytoplankton action limits for potential NST producing species in seawater samples collected from NSW shellfish aquaculture areas (NSW FA, 2014).

Phytoplankton species	Toxin	Trigger flesh sampling (cells/L)	Alert level – close harvest area pending flesh testing results	Issue public health warning (cells/L)
Karenia cf *brevis*	NST	50,000	500,000	N/A

During the open harvest status, NSW local shellfish programs collect routine samples for phytoplankton and biotoxin analysis in each oyster growing estuary in NSW to monitor for potential risks from toxic phytoplankton. Sampling requirements for shellfish aquaculture areas is outlined in the NSW MBMP. Sampling marine phytoplankton and biotoxins in accordance with the requirements set out in this plan is mandatory under the Australian Shellfish Quality Assurance Program (ASQAP) (ASQAAC, 2016) and the NSW Shellfish Industry Manual. The NSW Food Authority provide training to members of the local shellfish programs and only qualified samplers are permitted to collect monitoring samples. This monitoring is required to manage the potential health risks that toxic phytoplankton pose to consumers of shellfish. Fortnightly phytoplankton (seawater) and monthly biotoxin (shellfish flesh) sampling is the minimum requirement for NSW shellfish aquaculture areas under the open status.

All local shellfish programs are subject to the requirements in the NSW MBMP, however, the location and the number of sample sites in each harvest area represents the specific regional conditions. The results of the monitoring samples are actioned in near-real time. Reports on fortnightly algal sampling and monthly biotoxin results are assessed within two hours of receipt. In the case that a PAL has been exceeded or a positive biotoxin test result occurs, the relevant laboratories provide verbal notification to the NSW Food Authority who action the report immediately. The frequency of monitoring is increased to weekly following detection of phytoplankton cell concentrations above the specified trigger levels and/or reports of a positive biotoxin test result. If closure PAL triggers from seawater samples and/or a positive biotoxin result from shellfish flesh above the regulatory limit are reported, the harvest area is closed pending the outcome of subsequent testing (Figure 2).

Prior to 2012, Jellett Rapid Test (JRT) kits were the primary resource for screening algal toxins in shellfish flesh. While providing a fast and inexpensive screen for the three main algal toxin groups (AST, PSTs and DSTs) found in NSW coastal waters, the inability of the test to provide a measure of toxin levels was restrictive for industry. Quantitative testing capabilities were limited. One NSW laboratory offered analytical testing for AST and quantification of PSTs was carried out via mouse bioassay in South Australia. The nearest facility that could offer a complete toxin analysis service was based in New Zealand. With the logistical difficulties of transporting time-sensitive samples to another country, this option was impractical for routine use by Australian industry members.

During 2012, a tender for analytical shellfish biotoxin analyses was issued for a centralised laboratory, based in Sydney. The shift to quantitative analysis has improved risk management for harvest areas, as it is now possible to relate phytoplankton abundance to biotoxin concentrations. Microalgal Services has provided phytoplankton identification and enumeration services for the entire NSW oyster industry since 2004. Microalgal Services has historically provided data collation, storage, database maintenance and data retrieval services on phytoplankton data for the Food Authority and provides expert scientific advice on phytoplankton. Both laboratories have participated in internationally recognised proficiency testing and regularly attend international conferences and meetings to keep abreast of emerging threats to the shellfish industry.

All phytoplankton and biotoxin results are securely stored online in the NSW Food Authority's central database. Hard copy files are also maintained and securely stored. This system meets the necessary protocols for all Australian government agencies and ensures the safe storage and back-up facilities for data and information pertaining to NSW shellfish aquaculture areas. Phytoplankton and biotoxin data are reviewed yearly for each classified harvest area as part of the annual review process.

The current classification status of NSW shellfish aquaculture areas was developed during 2000-2005 when risk assessments were carried out to assess their sanitary status. The first assessment of biotoxin risk was also undertaken for each estuary. Routine phytoplankton and biotoxin monitoring is now an integral part of managing potential food safety risks from algal toxins in each of the NSW shellfish aquaculture areas. Data is reviewed each year as part of the annual and/or triennial review undertaken for each harvest area.

In 2009, the Food Authority contributed funding and expertise toward a PhD research project aimed at developing an improved understanding of phytoplankton diversity in NSW coastal waters. As part of the study, NSW Food Authority phytoplankton and biotoxin monitoring data for the period 2005-2009 were analysed. Phytoplankton data were examined for spatial and temporal trends in toxic events and abundance of toxic/nuisance species. This study investigated for any high and low risk estuaries/sites and the abundance and distribution of toxic phytoplankton within the oyster growing estuaries of New South Wales. The study was subject to peer review and has been published in the journal Environmental Monitoring and Assessment (Ajani et al., 2013).

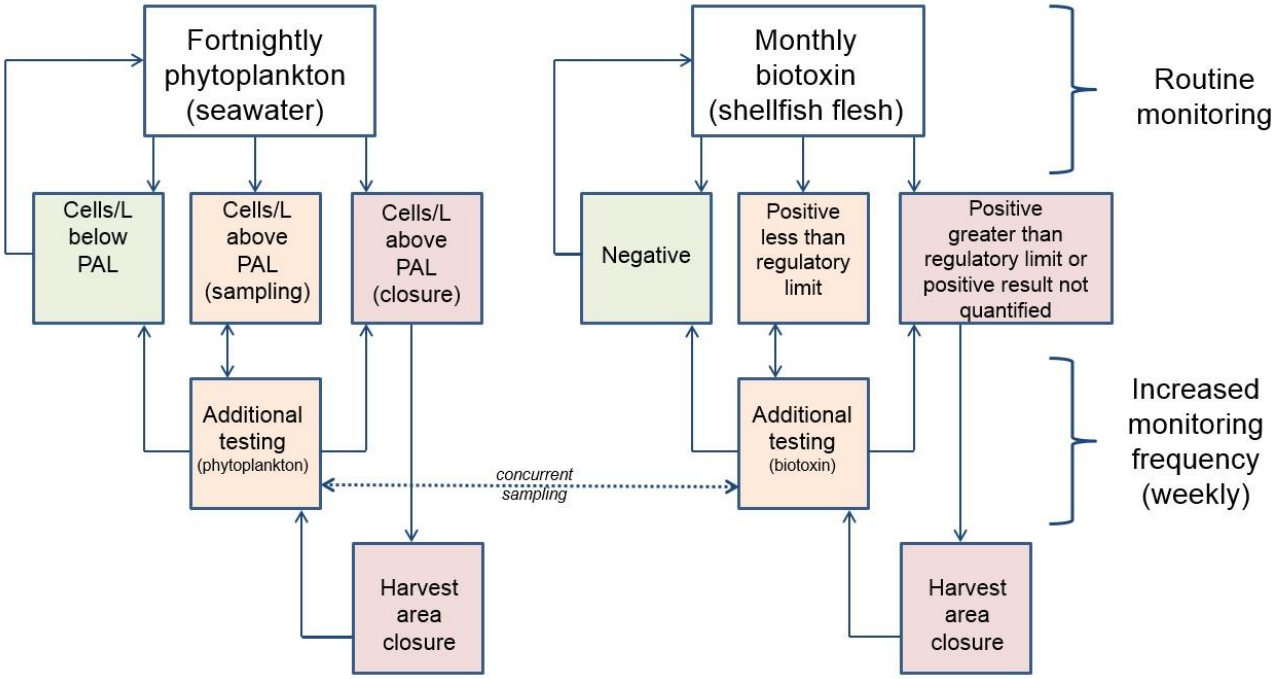

Figure 2. Summary of routine monitoring for phytoplankton and biotoxin samples under the NSW Marine Biotoxin Management Plan (NSW FA, 2014). Sampling frequency is increased following breach of phytoplankton action limit (PAL) triggers in seawater samples or positive biotoxin reports from shellfish flesh.

The evolution of the existing monitoring program now provides more than twelve years of information from which the risk to seafood consumers from potentially harmful phytoplankton species in aquaculture zones can be further assessed. This risk assessment has reviewed the historical phytoplankton and biotoxin data collected between January 2004 and December 2016 (Table 6). Harvest areas in Lake Conjola, Jervis Bay and Hunter River are not currently classified. Routine monitoring in these areas has not taken place since 2006 (Lake Conjola), 2008 (Jervis Bay) and 2009 (Hunter River). Phytoplankton and biotoxin data from these dormant areas was included for completeness, as these areas may return to aquaculture operations in the future. It is expected that Jervis Bay will return to operation during 2017. This will see the area return to a regular sampling program in line with the NSW MBMP.

Table 6 Summary of data included in current risk assessment.

Sample	Type	Total	Dates
Routine phytoplankton monitoring	Sea water	15,082	Jul 2005 – Dec 2016
AST	Shellfish flesh	8,147	Sept 2004 – Dec 2016
PSTs	Shellfish flesh	8,171	Jan 2004 – Dec 2016
DSTs	Shellfish flesh	8, 885	Jan 2004 – Dec 2016

Both the prevalence of phytoplankton in seawater samples and detection of biotoxins in shellfish tissue were used to determine the biotoxin food safety hazard for the state. The data were analysed in relation to the current regulatory limits for AST, DSTs and PSTs (Table 1) and the PAL triggers from the NSW MBMP (Tables 2-5). NSTs and AZTs have not been detected to date in NSW, however, species that have the potential to produce these toxins are considered as part of the risk assessment processes. Quantitative testing for biotoxins, where a value for toxin concentration in shellfish tissue (if present) is reported, rather than a positive or negative result only has been available since 2012 and is now used by the majority of local shellfish programs for routine testing.

Occurrence of potentially harmful phytoplankton species and detection of biotoxins in NSW shellfish aquaculture areas

Pseudo-nitzschia spp. (amnesic shellfish toxin)

Some species of *Pseudo-nitzschia* can be difficult to identify without the use of transmission electron microscopy (TEM). This is particularly relevant for smaller species. TEM is impractical for routine monitoring programs due to cost and a complex sample preparation process. Generally, when examining the samples for monitoring purposes via light microscopy, a cell width of up to 2.5-3 µm is used to characterise the *P. delicatissima* group, which can contain more than one species. Some species within the *P. delicatissima* group (e.g. *Pseudo-nitzschia galaxiae*) were distinguished by the phytoplankton identification laboratory. Larger *Pseudo-nitzschia* species can be more easily identified by suitably qualified laboratories based on other distinguishing cell features. Concentrations of *Pseudo-nitzschia multistriata* were evaluated as part of *P. delicatissima* group and separately (Table 7), as this species exceed 3 µm (2.2-3.9 µm), and is known to be toxin producing in the NSW region (Ajani et al., 2013).

Potentially harmful *Pseudo-nitzschia* spp. were detected in 6.5 - 56.4 % of discrete water samples between 2005 and 2016 (Table 7). Exceedances of PAL triggers by these species in seawater samples were ≤ 2 % for flesh testing and ≤ 0.1 % for harvest area closures (Table 7). Combined cell concentrations of non-*delicatissima* group *Pseudo-nitzschia* spp. exceeded the PAL flesh test limit 168 times and closure triggers were exceeded 9 times (Table 7). Not all species reported from the genus are confirmed to be toxin-producing in NSW/Australian waters (NSW FA, 2014), and the reports to species level enable a more informed risk management approach.

Positive AST results were reported in 2% (163) of samples across 17 (of 30) estuaries (Tables 8 and 9). As of December 2016, regulatory limits for biotoxins in *S. glomerata* have only been exceeded during one event. This bloom was extraordinary given the large cell concentration (> 6 million cells/L) of *Pseudo-nitzschia delicatissima* group in 2010 at Wagonga Inlet (Narooma). The causative species was confirmed by transmission electron microscopy (TEM) to be *Pseudo-nitzschia cuspidata* (Ajani et al., 2013). This event occurred following an extreme bush fire through the catchment that was quickly followed by extreme flooding, washing a significant amount of burnt material into the lake (A. Zammit, *pers comm*). During this event, PAL triggers for *P. cuspidata* in seawater samples were breached 17 (10 and 7) times over 2 phytoplankton monitoring sites in the estuary. During this event, AST concentrations ranged from 7-34 mg domoic acid (DA)/Kg, with 13 samples greater than the regulatory limit (20 mg/Kg) (Table 8). The Wagonga Inlet harvest areas were closed during the bloom and no illnesses were associated with this event. A trade level recall was enacted immediately upon receipt of the positive results and the harvest areas were placed under mandatory closure for 16 weeks.

Since the 2010 Wagonga bloom event, the largest cell concentrations of *Pseudo-nitzschia delicatissima* group were > 13 million cells/L (31 October 2011) at Tuross Lake. However, AST was not detected from JRT kits during this event. All other available quantified AST results were < 4.5 mg/Kg (regulatory limit = 20 mg/Kg). Positive AST detections across the state have been associated with *P. delicatissima* group, *Pseudo-nitzschia fraudulenta/australis, Pseudo-nitzschia pungens/multiseries, Pseudo-nitzschia multistriata, Pseudo-nitzschia subpacifica/heimii* and occasionally *Pseudo-nitzschia* cf. *galaxiae* (Table 10). *Pseudo-nitzschia turgidula/dolorosa* was also present at times when AST positive results were reported. Recently this group has been confirmed by the laboratory via TEM to be *Pseudo-nitzschia brasiliana*. This species is part of the '*americana*' group of *Pseudo-nitzschia* spp. and is generally not considered to be toxin producing; with only one report of toxin production in this

species recorded from Tunisian waters (Sahraoui et al., 2011). More than one toxin-producing species can be present at more than one time, and it was not possible to ascertain the relative contribution of each species to the toxin profile when low AST concentrations were reported. On occasion, extremely low AST positives have not been linked to a particular species (Table 10). It should be noted that since the routine availability of quantitative testing during 2012, AST positive results increased. This is primarily due the increased sensitivity of the LCMS-MS methods used. The majority (~77%) of quantified positive results between 2012 and 2014 (n=115) were less than 1% (0.2 mg/Kg) of the regulatory limit, and only 9 of the positive sample results during the same period were greater than 5% (1 mg/Kg) of the regulatory limit. Since 2015, the lower limit of reporting for AST was increased to 1 mg/Kg by the laboratory. This has significantly reduced testing costs for industry while still appropriately managing the risk of AST contamination in shellfish.

Table 7 Summary of detections of *Pseudo-nitzschia* spp. in NSW shellfish aquaculture areas 2005-2016 (n=15,082) in relation to PAL triggers for this genus in seawater samples to initiate biotoxin flesh testing or harvest area closure. Note: there is no PAL harvest area closure trigger for *P. delicatissima* group specified in the NSW MBMP (2014).

	***P. delicatissima* group**	***P. multistriata****
No of Detections	8,508	974
% of Total Samples	56.4	6.5
Maximum (cells/L)	13,300,000	730,000
Minimum (cells/L)	49	500
Mean (cells/L)	63,328	5,730
Median (cells/L)	3,500	2,000
PAL (cells/L, flesh test)	500,000	50,000
Total exceedance of PAL	259	10
% of Total Samples	1.72	0.07
PAL (cells/L, closure trigger)		500,000
Total exceedance of PAL		1
% of Total Samples		0.01%

Cont'd	*P. fraudulenta /australis*	*P. pungens /multiseries*	*P. subpacifica /heimii*	**Total *Pseudo-nitzschia* spp. (non-P. *delicatissima*)**
No of Detections	2,217	3,121	1,701	5,522
% of Total Samples	14.7	20.7	11.3	36.6
Maximum (cells/L)	1,200,000	770,000	700,000	1,200,000
Minimum (cells/L)	25	20	50	20
Mean (cells/L)	8,512	5,221	6,765	9,425
Median (cells/L)	2,000	2,000	2,000	2,300
PAL (cells/L, flesh test)	50,000	50,000	50,000	50,000
Total exceedance of PAL	64	32	23	168
% of Total Samples	0.42	0.21	0.15	1.11
PAL (cells/L, closure trigger)	500,000	500,000	500,000	500,000
Total exceedance of PAL	3	2	2	9
% of Total Samples	0.02	0.01	0.01	0.06

*This species may be considered as part of the *P. delicatissima* group (refer to text).

Table 8 Summary of AST testing and positive detections in shellfish flesh across all NSW shellfish aquaculture areas 2004-2016 (n=8,147).

	Amnesic Shellfish Toxin												
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
No of Samples	6	522	640	707	700	666	731	686	698	713	721	685	672
No of Detections	0	0	0	2	0	1	19	0	12	67	59	0	3
% of Total Samples	0	0	0	0.3	0	0.2	2.6	0	1.7	9.4	8.2	0.0	0.4
Maximum concentration (mg/Kg)*	0	0	0	nq	0	nq	34	0	0.13	4.5	1.6	0	2.7
Regulatory Limit (mg/Kg)	20	20	20	20	20	20	20	20	20	20	20	20	20
Total exceedance of Regulatory Limit	0	0	0	nq	0	nq	13	0	0	0	0	0	0
% of Total Samples	0	0	0	nq	0	nq	1.8	0	0	0	0	0	0

*where available, prior to 2012 routine quantitative analysis was not readily available

nq - quantitative data not available

Table 9 Summary of positive AST detections in shellfish flesh across NSW estuaries with shellfish aquaculture areas (2004-2016).

Wagonga Inlet was the only estuary where the regulatory limit was exceeded (see text). Maximum quantified AST results across other harvest areas did not exceed 4.5 mg/Kg DA (Table 8).

Estuary	Amnesic Shellfish Toxin													
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
Tweed River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Richmond River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarence River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wooli River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bellinger/Kalang Rivers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nambucca River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macleay River	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Hastings River	-	-	-	-	-	-	-	-	5	7	4	-	-	16
Camden Haven River	-	-	-	-	-	-	-	-	-	6	-	-	-	6
Manning River	-	-	-	-	-	1	-	-	-	4	2	-	-	7
Wallis Lake	-	-	-	-	-	-	-	-	3	5	12	-	-	20
Port Stephens	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hunter River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brisbane Water	-	-	-	-	-	-	-	-	-	1	1	-	1	3
Patonga River	-	-	-	-	-	-	-	-	1	-	1	-	-	2
Hawkesbury River	-	-	-	-	-	-	-	-	-	17	8	-	-	25
Georges River	-	-	-	-	-	-	-	-	-	2	3	-	-	5
Shoalhaven/Crookhaven Rivers	-	-	-	1	-	-	-	-	-	2	12	-	2	17
Jervis Bay	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lake Conjola	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clyde River	-	-	-	-	-	-	-	-	-	3	3	-	-	6
Tuross Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wagonga Inlet	-	-	-	-	-	-	18	-	-	19	11	-	-	48
Bermagui River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wapengo Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nelson Lagoon	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Merimbula Lake	-	-	-	1	-	-	1	-	-	-	-	-	-	2
Pambula Lake	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Twofold Bay	-	-	-	-	-	-	-	-	2	-	-	-	-	2
Wonboyn River	-	-	-	-	-	-	-	-	-	-	1	-	-	1

Note: Samples were not collected from all years from Hunter River, Jervis Bay and Lake Conjola (refer text).

Table 10 Summary of *Pseudo-nitzschia* spp. associated with positive AST detections in shellfish flesh (Table 9) across NSW estuaries.

Associations are based on available phytoplankton data from concurrent seawater samples. 'unconfirmed' refers to occasions where *Pseudo-nitzschia* spp. were not were not detected in concurrent samples.

Estuary	Species associated with AST positive biotoxin results, as determined from phytoplankton reports
Macleay River	*P. pungens/multiseries*
Hastings River	*P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii,* unconfirmed
Camden Haven River	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries*
Manning River	*P. delicatissima* group, *P. pungens/multiseries,* unconfirmed
Wallis Lake	*P. delicatissima* group, *P. fraudulenta/australis, P.* cf. *galaxiae*
Brisbane Water	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii,*
Patonga River	*P. delicatissima* group, *P. pungens/multiseries, P. multistriata, P. subpacifica/heimii,*
Hawkesbury River	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii, P. turgidula/dolorosa*
Georges River	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii, P. turgidula/dolorosa,* unconfirmed
Shoalhaven/Crookhaven Rivers	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii, P. turgidula/dolorosa,* unconfirmed
Clyde River	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii, P. turgidula/dolorosa,* unconfirmed
Wagonga Inlet	*P. delicatissima* group, *P. cuspidata* (confirmed by TEM, 2010), *P. fraudulenta/australis, P. pungens/multiseries, P. multistriata, P. subpacifica/heimii, P.* cf. *galaxiae*
Nelson Lagoon	*P. delicatissima* group, *P. multistriata, P. subpacifica/heimii*
Merimbula Lake	*P. delicatissima* group, *P. fraudulenta/australis, P. pungens/multiseries*
Pambula Lake	*P. pungens/multiseries, P. subpacifica/heimii*
Twofold Bay	*P. delicatissima* group
Wonboyn River	*P. delicatissima* group, *P. pungens/multiseries*

P. turgidula/dolorosa in NSW samples has recently been confirmed by TEM to be *P. brasiliana*, which is generally not considered to be toxin producing (refer to text).

Alexandrium spp. and *Gymnodinium catenatum* (paralytic shellfish toxins)

Alexandrium species identified in the southeastern waters of Australia include;

- *Alexandrium affine**,
- *Alexandrium catenella* Group IV ribotype (= *Alexandrium pacificum*)*,
- *Alexandrium diversaporum*,
- *Alexandrium fraterculus*,
- *Alexandrium margalefi*,
- *Alexandrium minutum**,
- *Alexandrium ostenfeldii**,
- *Alexandrium pseudogonyaulax*,
- *Alexandrium tamarense* Group V ribotype (= *Alexandrium australiense*)*

(Ajani et al., 2013; Farrell et al., 2013; Hallegraeff et al., 2010; Hallegraeff et al., 1991; John et al., 2014; Murray et al., 2014; Murray et al., 2012).

Species marked with * are known to be PST-producing (Anderson et al., 2012). Note: *A. affine* is mentioned above as a potentially toxic species. This species was not reported from discrete seawater sample counts in samples from NSW aquaculture areas. *Alexandrium fundyense* (previously *A. tamarense* Group I ribotype)* has been detected in Tasmanian waters but its presence has not been identified in NSW to date (Ajani et al., 2017). *Gymnodinium catenatum* is also PST-producer known to be present in the region. However, this species has not been linked to biotoxin positives in NSW shellfish aquaculture areas to date. Of the *Alexandrium* species known to be toxin producing, *A. catenella* (Group IV) occurs most frequently in NSW shellfish aquaculture areas (Table 11). Risk management decisions are supported by provision of reports of species that are not known to produce toxins. From the available quantitative data, PST detections did not exceed the regulatory limit of 0.8mg/Kg until late 2016 (Table 12).

In phytoplankton samples collected 18 October 2016, a bloom of *Alexandrium* was detected in concentrations up to 450 cells/L within Twofold Bay (testing PAL for this species is 200 cells/L). A biotoxin sample collected on the same day was positive for PSTs (0.78 mg/Kg total PSTs). The Twofold Bay harvest areas were within a seasonal closure at the time the positive toxin result was detected. As these results were close to the regulatory limit for PSTs, the local program agreed to undertake further testing prior to the harvest area reopening. Additional samples collected 24 October 2016 showed that toxin levels had increased (5.4 mg/Kg total PSTs) and *Alexandrium* concentrations had increased to a maximum of 89,000 cells/L.

Species within the *Alexandrium tamarense/catenella/fundyense* species complex are morphologically similar but their toxicological profiles vary. In the phytoplankton samples collected 18 October 2016, the *Alexandrium* species present was reported to resemble *Alexandrium fundyense.* These algae were present as single cells rather than in short chains and were larger with a more robust appearance than *A. catenella*, which is routinely observed in NSW. As the bloom progressed, the samples were reported to contain a mix of both (the uncommon *A. fundyense* and the common *A. catenella*) cell types. These differences in cell characteristics could be attributed to physiological

changes in the more common *A. catenella* cells depending on the stage of the bloom, which is unknown prior to 18 October 2016. There were no phytoplankton or biotoxin data available dating back to July 2016 as the harvest areas were within a seasonal closure. Preliminary results of genetic testing in a sample collected in Twofold Bay on 24 October 2016 did not detect *A. fundyense*. These results and a full description of the Twofold Bay bloom event will be presented in a manuscript which is currently in preparation. If a bloom of this type was to reoccur, targeted genetic testing could determine whether more than one *Alexandrium* species is present.

The maximum concentration of PSTs was 7.2 mg/Kg (21 November 2016). The harvest areas remained closed until 16 December 2016 when the bloom declined and toxin levels had decreased to below the regulatory limit for two consecutive samples one week apart. Public health warnings were issued through the relevant media channels. No illnesses were reported due to consumption of shellfish from Twofold Bay during this event. While the highest cell concentrations of *Alexandrium* were reported from phytoplankton sampling sites in Twofold Bay, evidence of the bloom was detected in other south coast shellfish harvest areas. There were elevated concentrations (1,200-15,000 cells/L) of *Alexandrium* detected along the coastal shelf up to 13 km north and 21 km south of Twofold Bay. During the bloom event, PST levels did not exceed the regulatory limit in other NSW shellfish harvest areas. The current biotoxin sampling program was effective as no shellfish exceeding the regulatory limits for PST were marketed.

Apart from the 2016 bloom event, the majority of PST positives have been reported from central NSW (Table 13). *Alexandrium* spp. cyst beds are known to be present from lower Hawkesbury River to Batemans Bay (Hallegraeff et al., 1998; Lincoln-Smith and Smith, 1993), but *Alexandrium* cysts have also been detected in other major shipping ports on the NSW Coast (Eden (Twofold Bay): CSIRO (1997), Newcastle: CSIRO (1998)).

Detections of positive PSTs across the state have been associated with *A. catenella, A. minutum, A. tamarense and A. ostenfeldii* (Table 14). More than one toxin-producing species can be present at more than one time. However, from the available data, it appears that there is a north-south distribution pattern of *A. tamarense*, *A. minutum*, *A. catenella* and *A. ostenfeldii*. The reports of 'unconfirmed' species in Table 14 suggested that any bloom had ceased or dispersed prior to the biotoxin sample collection. Given that *Alexandrium i*s a cyst-forming species it is likely that the same species were responsible for the positive biotoxins. Prior to the availability of quantitative data, the harvest area was closed until negative biotoxin tests were confirmed. Sampling frequency was changed to weekly phytoplankton and biotoxin test following all detections of positive quantified PST results below the regulatory limit, and a consistent decline in toxicity is monitored. On occasions where low PST positives were not linked to one specific species (Table 10), maximum PST levels did not exceed 33% of the regulatory limit and these events did not usually surpass 2-3 weeks.

Table 11 Summary of detections of *Alexandrium* spp. in NSW shellfish aquaculture areas 2005-2016 (n=15,082) in relation to PAL triggers for this genus in seawater samples to initiate biotoxin flesh testing or harvest area closure.

	A. catenella/ fundyense	*A. fraterculus*	*A. insuetum*	*A. margalefi*	*A. minutum*
No of Detections	614	83	23	371	295
% of Total Samples	4.1	0.5	0.2	2.5	2.0
Maximum (cells/L)	89,000	2,600	150	2,300	3,900
Minimum (cells/L)	20	17	50	20	20
Mean (cells/L)	967	202	70	97	165
Median (cells/L)	75	100	50	50	50
PAL (cells/L, flesh test)	200	200	200	200	200
Total exceedance of PAL	159	21	-	43	39
% of Total Samples	1.05	0.14	0.00	0.28	0.26
PAL (cells/L, closure trigger)	500	500	500	500	500
Total exceedance of PAL	70	7	-	4	21
% of Total Samples	0.46	0.05	0.00	0.03	0.14

cont'd	***A.***	***A.***	***A***	**Total**

	ostenfeldii	*pseudogonyaulax*	*tamarense*	*Alexandrium* spp.
No of Detections	261	984	147	2,682
% of Total Samples	1.7	6.5	1.0	17.8
Maximum (cells/L)	1,100	3,000	11,000	89,000
Minimum (cells/L)	20	10	25	17
Mean (cells/L)	97	157	285	381
Median (cells/L)	50	75	50	100
PAL (cells/L, flesh test)	200	200	200	200
Total exceedance of PAL	26	227	26	676
% of Total Samples	0.17	1.50	0.17	4.48
PAL (cells/L, closure trigger)	500	500	500	500
Total exceedance of PAL	7	51	10	228
% of Total Samples	0.05	0.34	0.07	1.51

The grouping of *A. catenella/fundyense* is reported by the phytoplankton laboratory but *A. fundyense* has not been confirmed to be present in NSW shellfish aquaculture areas to date (refer to text).

Table 12 Summary of PST testing and positive detections in shellfish flesh across all NSW shellfish aquaculture areas 2004-2016 (n=8,1017).

	Paralytic Shellfish Toxins												
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
No of Samples	18	577	640	709	699	679	739	683	688	698	717	692	692
No of Detections	6	3	6	9	1	21	28	6	10	5	11	6	22
% of Total Samples	33	0.6	0.9	1.3	0.1	3.1	3.8	0.9	1.5	0.7	1.5	0.9	3.2
Maximum concentration (mg/Kg) *	nq	nq	nq	nq	nq	nq	nq	nq	0.66	0.13	0.16	0.26	7.2
Regulatory Limit (mg/Kg)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Total exceedance of Regulatory Limit	nq	nq	nq	nq	nq	0	nq	nq	0	0	0	0	9
% of Total Samples	nq	nq	nq	nq	nq	0	nq	nq	0	0	0	0	1.3

*where available, prior to 2012 routine quantitative analysis was not readily available
nq - quantitative data not available

Table 13 Summary of positive PST detections in shellfish flesh across NSW estuaries with shellfish aquaculture areas (2004-2016).

Twofold Bay was the only estuary where the regulatory limit was exceeded (see text). Maximum quantified PST results across other harvest areas did not exceed 0.66 mg/Kg STX (Table 12).

	Paralytic Shellfish Toxins													
Estuary	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**Total**
Tweed River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Richmond River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarence River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wooli River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bellinger/Kalang Rivers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nambucca River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macleay River	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Hastings River	-	-	-	-	-	-	1	-	1	-	-	-	-	3
Camden Haven River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Manning River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wallis Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Port Stephens	-	-	1	-	-	6		-	3	-	-	1	-	11
Hunter River	-	-	1	1	-	-	-	-	-	-	-	-	-	2
Brisbane Water	-	-	1	-	-	4	13	-	-	1	1	-	-	20
Patonga River	-	-	-	1	-	9	3	-	-	-	-	-	-	13
Hawkesbury River	-	-	-	6	-	1	1	2	-	1	3	-	1	15
Georges River	3	2	1	-	-	-	3	3	4	1	2	1	-	20
Shoalhaven/ Crookhaven Rivers	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Jervis Bay	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lake Conjola	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Clyde River	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Tuross Lake	-	-	-	-	-	-	-	-	-	-	3	-	-	3
Wagonga Inlet	2	-	-	-	-	-	7	-	-	-	-	-	2	11
Bermagui River	-	-	1	-	-	1	-	-	-	-	-	2	-	4
Wapengo Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nelson Lagoon	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Merimbula Lake	-	-	-	1	-	-	-	-	-	-	1	-	-	2
Pambula Lake	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Twofold Bay	-	-	-	-	-	-	-	-	2	-	-	-	18	20
Wonboyn River	-	-	-	-	-	-	-	1	-	2	-	1	-	4

Note: Samples were not collected from all years from Hunter River, Jervis Bay and Lake Conjola (refer to text).

Table 14 Summary of *Alexandrium* spp. associated with positive PST detections in shellfish flesh (Table 13) across NSW estuaries. Associations are based on available phytoplankton data from concurrent seawater samples. 'unconfirmed' refers to occasions where potentially toxic *Alexandrium* spp. were not detected in concurrent samples.

Estuary	Species associated with PST positive biotoxin results, as determined from phytoplankton reports
Macleay River	Unconfirmed
Hastings River	*A. tamarense*, *A. minutum*
Port Stephens	*A. minutum*, unconfirmed
Hunter River	Unconfirmed
Brisbane Water	*A. catenella/fundyense*, unconfirmed
Patonga River	*A. catenella/fundyense*
Hawkesbury River	*A. catenella/fundyense*, *A. minutum*, unconfirmed
Georges River	*A. catenella/fundyense*, unconfirmed
Shoalhaven/Crookhaven Rivers	Unconfirmed
Tuross Lake	Unconfirmed
Wagonga Inlet	*A. catenella/fundyense*, unconfirmed
Bermagui River	Unconfirmed
Nelson Lagoon	Unconfirmed
Merimbula Lake	Unconfirmed
Pambula Lake	*A. catenella/fundyense*
Twofold Bay	*A. catenella/fundyense*, A. ostenfeldii, unconfirmed
Wonboyn River	A. ostenfeldii, unconfirmed

The grouping of *A. catenella/fundyense* is reported by the phytoplankton laboratory but *A. fundyense* has not been confirmed to be present in NSW shellfish aquaculture areas to date (refer to text).

Occurrences of *G. catenatum* have been rare in NSW shellfish aquaculture areas, with maximum cell concentrations up to 5,100 cells/L reported in 22 (of 15,082) samples (Table 15). To date, *G.catenatum* has not been linked to detection of PSTs in NSW shellfish samples. Maximum concentrations (5,100 cells/L) were observed in Brisbane Water (2012). Other exceedances of the flesh testing trigger were in Nelson Lagoon (2006, 2012) and Tuross (2007). Remaining reports detected ≤ 450 cells/L.

Table 15 Summary of detections of *Gymnodinium catenatum* in NSW shellfish aquaculture areas 2005-2016 (n=15,082) in relation to PAL triggers for this species in seawater samples to initiate biotoxin flesh testing or harvest area closure.

	Gymnodinium catenatum
No of Detections	26
% of Total Samples	0.17
Maximum (cells/L)	5,100
Minimum (cells/L)	25
Mean (cells/L)	738
Median (cells/L)	165
PAL (cells/L, flesh test)	1,000 (mussels)/2,000 (other shellfish)
Total exceedance of PAL	5
% of Total Samples	0.03
PAL (cells/L, closure trigger)	5,000
Total exceedance of PAL	1
% of Total Samples	0.01

Dinophysis spp. and *Prorocentrum lima* (diarrhetic shellfish toxins)

Traditionally, DSTs included okadaic acids, dinophysistoxins, yessotoxins and pectenotoxins and when detected and quantified, their concentrations have been combined. Pectenotoxin-2 seco acid was not included in total DSTs as this pectenotoxin analogue is considered non or extremely low toxicity (Miles et al., 2004). Separate consideration of these toxins is now possible due to improved technologies for their detection.

With regards to okadaic acid, the main sources are *Dinophysis* spp. Also, *Dinophysis* spp. are the only known source of pectenotoxins (Reguera et al., 2014). Some species of *Prorocentrum* are known to produce DSTs. Foden et al. (2005) demonstrated toxicity (okadaic acid and dinophysistoxins) in the epibenthic *Prorocentrum lima*. Under the NSW MBMP guidelines, this species has a PAL of 500 cells/L (Table 4). This value has been exceeded eight times in 0.05% of phytoplankton samples (Table 16). To date, *P. lima* has not been associated with positive DSTs in NSW shellfish aquaculture areas. Maximum concentrations have been reported from Wonboyn Lake (2,150 cells/L) and Camden Haven (1,600 cells/L) estuaries. Overall cell concentrations have been low (mean = 104 cells/L, median = 50 cells/L (Table 16)).

Dinophysis acuminata and *Dinophysis caudata* were the most frequently observed members of the DST-producing genus *Dinophysis*, although generally cell numbers are low (Table 17). Maximum cell concentrations have been reported from Camden Haven during 2014 (33,000 cells/L, *D. acuminata*). However, DSTs were not detected in oyster samples collected during this event. Similarly, 22,000 cells/L *D. acuminata* were reported from the Manning River during January 2015, while biotoxin samples collected at the same time were negative. From the available quantitative data, DSTs have never exceeded the regulatory limit of 0.2 mg/Kg in classified shellfish aquaculture areas (Table 18). Positive DSTs have been reported from 10 (of 30) estuaries (Table 19) and have been mostly associated with *D. acuminata* (Table 20).

To date, there is no evidence that yessotoxins and pectenotoxins are toxic to humans, although acute toxicity has been demonstrated in animals (Lawrence et al., 2011; Munday and Reeve, 2013). Pectenotoxins have a different chemical structure and mechanism of action (okadaic acid is diarrheagenic while pectenotoxins are not) (Alexander et al., 2008). The Panel on Contaminants in the Food Chain (CONTAM Panel) have recommended that pectenotoxins be regulated separately from okadaic acid (Alexander et al., 2009). Five reports of low-level pectenotoxin-2 (max 0.063 mg/Kg) were reported from Wonboyn Lake during 2014 and 2016 (Tables 18 and 19).

Yessotoxin screening is part of the routine lipophilic shellfish toxin screen conducted by the biotoxin laboratory. To date there has only been one positive detection of yessotoxin (0.05 mg/Kg) in NSW from targeted sampling of *M. edulis* from Sydney Fish Markets (source Twofold Bay) during 2015. This is an extremely low level (European regulatory limit for yessotoxin = 1mg/Kg).

Table 16 Summary of detections of *Prorocentrum lima* in NSW shellfish aquaculture areas 2005-2016 (n=15,082) in relation to PAL triggers for this species in seawater samples to initiate biotoxin flesh testing or harvest area closure.

	Prorocentrum lima
No of Detections	181
% of Total Samples	1.69
Maximum (cells/L)	2,150
Minimum (cells/L)	25
Mean (cells/L)	104
Median (cells/L)	50
PAL (cells/L, flesh test)	500
Total exceedance of PAL	8
% of Total Samples	0.05

Table 17 Summary of detections of *Dinophysis* spp. in NSW shellfish aquaculture areas 2005-2016 (n=15,082) in relation to PAL triggers for this genus in seawater samples to initiate biotoxin flesh testing.
Note: there is no PAL harvest area closure trigger for *Dinophysis* spp. specified in the NSW MBMP (2014).

	D. acuminata	*D. acuta*	*D. caudata*	*D. fortii*	*D. hastata*
No of Detections	2811	35	858	54	5
% of Total Samples	18.6	0.2	5.7	0.4	0.03
Maximum (cells/L)	33,000	190	4,100	550	50
Minimum (cells/L)	10	25	20	20	50
Mean (cells/L)	273	70	174	80	50
Median (cells/L)	100	50	100	50	50
PAL (cells/L, flesh test)	1000	500	500	500	500
Total exceedance of PAL	128	0	51	1	0
% of Total Samples	0.85	0.00	0.34	0.01	0.00

Cont'd	*D. mitra*	*D. rotundata*	*D. tripos*	Total *Dinophysis* spp.
No of Detections	11	253	146	3,694
% of Total Samples	0.1	1.7	1.0	24.5
Maximum (cells/L)	125	500	1,500	33,050
Minimum (cells/L)	25	20	20	10
Mean (cells/L)	55	82	115	261
Median (cells/L)	50	50	50	100
PAL (cells/L, flesh test)	500	500	500	500
Total exceedance of PAL	0	2	6	353
% of Total Samples	0.00	0.01	0.04	2.34

Table 18 Summary of DST testing and positive detections in shellfish flesh across all NSW shellfish aquaculture areas 2004-2016 (n=5,885).

	Diarrhetic Shellfish Toxins												
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
No of Samples	1	5	5	9	341	666	708	685	683	711	714	684	671
No of Detections	1	3[#]	0	5[#]	2	0	0	5	0	11	3^	0	2^
% of Total Samples	100	60	0	63	0.6	0	0	0.7	0	1.5	0.4	0	0.3
Maximum concentration (mg/Kg)*	nq	0.02 ^	0	0.02[$]	0.02[$]	0	0	nq	0	0.046	0.063^	0	0.036^
Regulatory Limit (mg/Kg)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total exceedance of Regulatory Limit	nq	0	0	0	0	0	0	nq	0	0	0	0	0
% of Total Samples	nq	0	0	0	0	0	0	nq	0	0	0	0	0

*where available, prior to 2012 routine quantitative analysis was not readily available
nq - quantitative data not available
#Pectenotoxin 2 seco acids also detected; ^Pectenotoxin 2 positive only; [$]Dinophysistoxin 3 positive

Table 19 Summary of positive DST detections in shellfish flesh across NSW estuaries that contain shellfish aquaculture areas 2004-2016. All positive reports were less than 30% of the regulatory limit.

Estuary	Diarrhetic Shellfish Toxins													
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
Tweed River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Richmond River	-	-	-	-	2	-	-	1	-	-	-	-	-	3
Clarence River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wooli River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bellinger/Kalang Rivers	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nambucca River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macleay River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hastings River	-	-	-	2	-	-	-	-	-	-	-	-	-	2
Camden Haven River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Manning River	-	-	-	1	-	-	-	2	-	4	-	-	-	7
Wallis Lake	1	1	-	2	-	-	-	-	-	-	-	-	-	4
Port Stephens	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hunter River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brisbane Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Patonga River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hawkesbury River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Georges River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shoalhaven/ Crookhaven Rivers	-	-	-	-	-	-	-	-	-	2	-	-	-	2
Jervis Bay	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lake Conjola	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Clyde River	-	-	-	-	-	-	-	-	-	2	-	-	-	2
Tuross Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wagonga Inlet	-	-	-	-	-	-	-	-	-	3	-	-	-	3
Bermagui River	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wapengo Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nelson Lagoon	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Merimbula Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pambula Lake	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Twofold Bay	-	1	-	-	-	-	-	2	-	-	-	-	-	3
Wonboyn River	-	-	-	-	-	-	-	-	-	-	3^	-	2^	5

Note: Samples were not collected from all years from Hunter River, Jervis Bay and Lake Conjola (refer text).
^Positive detections of DSTs in Wonboyn Lake during 2014 and 2016 were pectenotoxin-2 only.

Table 20 Summary of *Dinophysis* spp. associated with positive DST detections in shellfish flesh (Table 19) across NSW estuaries.

Associations are based on available phytoplankton data from concurrent water samples. 'unconfirmed' refers to occasions where potentially toxic *Dinophysis* spp. were not detected in concurrent samples.

Estuary	Species associated with DST positive biotoxin results, as determined from phytoplankton reports
Richmond River	*D. acuminata*
Hastings River	*D. acuminata*
Manning River	*D. acuminata*
Wallis Lake	*D. acuminata*, unconfirmed
Shoalhaven/Crookhaven Rivers	unconfirmed
Lake Conjola	unconfirmed
Clyde River	unconfirmed
Wagonga Inlet	*D. acuminata*
Twofold Bay	*D. acuminata*, unconfirmed
Wonboyn River	*D. caudata*

Karenia spp. and other potential neurotoxic shellfish toxin producers

Karenia brevis is the primary causative agent of NST. *K. brevis* has not been identified in the NSW monitoring program data to date. Early laboratory reports noted this species but further examination determined that the species was *Karenia papilionacea.* During 1993, 180 shellfish poisoning cases were reported from New Zealand. The event was originally reported to be caused by species similar to *K. brevis* that were later confirmed as an assemblage of *Karenia* spp. (*Karenia bicuneiformis*, *Karenia brevisulcata*, *Karenia papilionacea*, *Karenia seliformis*) (Haywood et al., 2004). Toxicity of *K. papilionacea* was below the limit of detection (< 0.0005 PbTX2 pg/cell) in a study by McNabb et al. (2006), however recent work by Fowler et al. (2015) suggests that this species can produce brevetoxins (NSTs). The relative toxicity of *K. papilionacea* is much lower when compared to *K. brevis*. In the study by Fowler et al. (2015) the average toxicity of *K. papilionacea* was 5 fg PbTX2/cell (0.005 fg PbTX2/cell), while McNabb et al. (2006) reported toxicity of *K. brevis* at 23 pg PbTX2/cell.

Production of brevetoxin or brevetoxin-like compounds, has been found within the genus *Chatonella, Fibrocapsa japonica* and *Heterosigma akashiwo* (Botana, 2014). However, these species have been primarily associated with fish kills and shellfish mortality events (Landsberg, 2002). NST-testing does not routinely take place in the current sampling program. However, monitoring of all potential NST-producing species is a customary part of the standard phytoplankton analysis.

Azadinium spp.

The current biotoxin screening methods include testing for at least three azaspiracid analogs. To date, no positive samples have been reported from routine biotoxin testing.

The first reported illness from AZTs occurred from consumption of Irish shellfish in 1995 (McMahon and Silke, 1996). Despite frequent detections of AZTs in European shellfish, the causative organism was difficult to determine due to its small size and similarities to other non-toxic species. The precise description of the AZT-producing dinoflagellate group *Azadinium* did not occur until 2009 (Tillmann et al., 2009). Subsequently, reports of the genus *Azadinium* have increased worldwide. The first report of detection of AZTs in New Zealand shellfish occurred in 2011 (Moisan et al., 2014). While AZTs have not been detected in NSW shellfish to date, toxin screening coupled with molecular tools would aid risk management should the need arise. Pyrosequencing (multiple genetic screening) of phytoplankton samples from Wagonga Inlet during 2012 detected *Azadinium* spp. (Kohli et al., 2014). Advances in molecular tools would aid risk assessment if AZAs were to be detected in NSW shellfish, as the causative species is extremely difficult to identify by traditional microscopy.

Hazard characterization

'The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable.'

Adverse health effects from the three main algal toxin groups found in NSW coastal waters are summarised in Table 21, and can range from mild illness to death in extreme cases of exposure to PSTs. There is limited data available on the long-term effects of exposure to low levels of PSTs and AST. However, chronic exposure to DSTs has been associated with tumours in the digestive system.

Table 21 Summary of clinical symptoms associated with the three main algal toxin groups found in NSW coastal waters (adapted from Table 1.2 in Hallegraeff (2003).

			Symptoms			
Toxin	**Onset time**	**Case**	**Nausea, vomiting, diarrhoea**	**Abdominal cramps/pain**	**Headache, dizziness**	**Tingling/numbness of face and neck /prickly sensation in fingers and toes**
AST	After 3-5 hours	Mild	x	x		
		Extreme	Decreased reaction to deep pain, dizziness, hallucinations, confusion, short term memory loss, seizures.			
PSTs	Within 30 mins	Mild	x		x	x
		Extreme	Muscular paralysis, respiratory difficulty, choking sensation, death through respiratory paralysis (2 - 24 hours after ingestion).			
DSTs	After 30 mins to a few hours (< 12 hours)	Mild	x	x		
		Extreme	Chronic exposure linked to tumour formation in the digestive system.			

The acute reference doses (ARfDs) provided by EFSA (2009) for AST, DST and PSTs were used to calculate the maximum concentration of shellfish toxin present in a 63 g portion of shellfish (median mollusc consumers in Australia, Anon. (2015)) to avoid exceeding the ARfD for each particular toxin (Table 22). The same evaluation process was also applied to one standard seafood serving (150 g: Australian adult 70 Kg,(FSANZ, 2011)), which is also comparable to the Australian high consumption data for molluscs (146 g: 90th percentile consumers, Anon. (2015)) (Table 22). These calculations demonstrated that the current regulatory limits for AST and DSTs were sufficiently protective for median shellfish consumers but indicated there was a potential higher risk of PST exposure for median consumers and a potential higher risk of exposure for all three toxin groups for high level shellfish consumers. These findings were similar to those of EFSA (2009) who focused on high level consumers (400 g portion), however, there is little epidemiological evidence to suggest that the current regulatory limits for these toxin groups are ineffective (Lawrence et al., 2011; Pointon et al., 2009).

Table 22 Exposure levels for consumers based on current Australian regulatory limits for AST, PSTs and DSTs and the acute reference doses (ARfDs) provided by EFSA (2009).

Toxin	Australian limit (mg/Kg) Standard 1.4.1, clause 3 of the Food Standards Code	Acute Reference Dose (ARfD) mg/Kg body weight (EFSA, 2009)	Max. concentration of shellfish toxin (mg/Kg) to avoid exceeding ARfD from 63 g portion (70 kg person)	Max. concentration of shellfish toxin (mg/Kg) to avoid exceeding ARfD from 150 g portion (70 kg person)
AST	20 DA	0.03 DA	33.3 DA	14.0 DA
PSTs	0.8 STX equiv.	0.0005 STX equiv.	0.56 STX equiv.	0.23 STX equiv.
DSTs - OA	0.2 OA equiv.	0.0003 OA equiv.	0.33 OA equiv.	0.14 OA equiv.
DSTs - PTX	0.2 OA equiv.	0.0008 PTX 2 equiv.	0.89 PTX2 equiv.	0.37 PTX2 equiv.

Note: Okadaic acid (OA), dinophysistoxins and pectenotoxins (PTX), current regulation specifies a combination; however, the CONTAM Panel concluded that PTX should be considered separately.

The ARfD of a chemical is equivalent to the no observable effect level (Lawrence et al., 2011). The establishment of an ARfDs usually includes a margin of error or variation. In the case of PSTs and DSTs this is three-fold, while the EFSA ARfD for AST includes an uncertainty factor of 30 to the lowest observable adverse effect level (LOAEL) of 0.9 µg/kg DA (EFSA, 2009).

A preliminary ARfD of 0.1 mg DA/Kg body weight was derived following investigation of the first AST illness outbreak in Canada (1987). This ARfD was used to establish the existing regulatory limit of 20 mg/Kg (Lawrence et al., 2011). Substituting this preliminary ARfD in lieu of the EFSA ARfD results in the maximum concentration of AST in 63 g and 150 g portions to be substantially higher than the regulatory limit at 111 mg/Kg and 46 mg/Kg, respectively. Since the establishment of the 20 mg/Kg regulatory limit for AST there has been no reported AST outbreaks in Canada. This limit is used by shellfish control agencies around the world, and with effective monitoring further AST outbreaks should not occur (Lawrence et al., 2011).

For PSTs, the application of an alternative ARfD of 0.007 mg STX equiv./kg body weight (FAO/IOC/WHO, 2004) would increase the maximum concentration of PSTs in 63 g and 150 g portions of shellfish to 0.78 mg/Kg and 0.33 mg/Kg, respectively. Generally, illness outbreaks linked to elevated PSTs have been associated with concentrations that greatly exceed the regulatory limit (e.g. > 2.5 mg/Kg). A recommendation of the Codex Committee on Fish and Fishery Products is that "*the long history of success (nearly 50 years) using an action level of 0.8 mg/kg with the mouse bioassay, with no human illnesses from commercially harvested product*" (CCFFP, 2006). There have been some cases of illness linked to illegal harvest from closed areas or in locations that were previously unaffected by PSTs (Lawrence et al., 2011). Similarly, the impact of DSTs on public health has been minimal since the establishment of regulatory limits (Lawrence et al., 2011; Pointon et al., 2009).

Exposure assessment

'The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.'

Prevalence of algal toxin producers in NSW.

The only potential source for NSW shellfish to accumulate algal toxins is from naturally occurring phytoplankton blooms within NSW aquaculture growing areas. While the macroalgal species *Jania* and *Ecklonia* have been suggested as sources of PSTs, this has not been confirmed in Australian waters to date (McLeod et al., 2010). Based on the Cawthron report (Todd et al., 2001), potentially toxic species have been reported from other Australian states. The potential risk of exposure of NSW shellfish consumers to algal toxins from Australian shellfish produced outside of NSW are managed by the relevant state shellfish control agency for each jurisdiction and is outside the scope of this assessment. This also applies to shellfish produce imported from other countries to NSW.

Reports of phytoplankton blooms in NSW coastal waters have primarily been due to water discolorations of non-toxic species. Two review papers summarising available data from 1890-1999 (Ajani et al., 2001) and 2000-2009 (Ajani et al., 2011) found that reports of phytoplankton blooms in the region have increased, although this may be due to the ad hoc nature of the blooms being reported. With respect to potentially harmful phytoplankton blooms, greater instances occurred during the period 1990-1999, than 2000-2009. To date, there have been two documented illness outbreaks following consumption of shellfish contaminated with biotoxins in pipis harvested from NSW coastal waters. Both cases were associated with diarrhetic shellfish toxins (DSTs). One outbreak of 59 cases occurred in 1997 (Ballina) and the other outbreak of 23 cases in 1998 (Newcastle) (Ajani et al., 2001).

This report assessed 22,203 shellfish flesh samples analysed for algal biotoxins collected between January 2004 and December 2016. During this same time period, only 13 (AST) and 9 (PST) samples (0.1% of total samples) exceeded regulatory limits. No illnesses in seafood consumers were reported from either event. The positive samples were associated with one unusual AST bloom event (Wagonga Inlet (Narooma) described earlier) which occurred during atypical environmental conditions, and a PST bloom event that caused high toxin concentrations in blue mussel samples collected from Twofold Bay, Eden.

While there is evidence to suggest that EFSA derived ARfDs may be exceeded in some cases (refer Table 22), the risk of human illness from algal toxins following consumption of NSW shellfish aquaculture produce is low. This is based on infrequent algal bloom events during the past twelve and a half years and the application of a rigorous routine monitoring program, which includes closure of harvest areas if reporting of results exceeds reporting time frames. The current algal and biotoxin monitoring program is an effective and necessary measure to avert human exposure to biotoxins. While exceedances of the regulatory limits for algal toxins in NSW has been rare, a historical low frequency of algal events does not mean that blooms and/or toxic events will not occur in the future.

NSW shellfish aquaculture production and consumer intake of shellfish.

Consumption data indicates that mollusc consumption is low across Australia in general. Mollusc consumption data by the 2+ years population age group were recently presented by the Williamtown Contamination Expert Panel (Anon., 2015), derived from 2011-12 National Nutrition and Physical Activity Survey (NNPAS) component of the 2011-13 Australian Health Survey. Three levels of mollusc consumption (kg day^{-1}) are presented (Table 23): mean mollusc consumption by all respondents (consumers and non-consumers) and median and 90th percentile mollusc consumption for consumers only (as shellfish may be consumed in larger proportions by certain groups e.g. shellfish farmers and their families) (Anon., 2015).

Table 23 Mollusc consumption data for the 2+ years population age group.

Data were based on Day 1 of the NNPAS 24 hour dietary recall survey (Anon., 2015).

			Mollusc consumption (Kg/day)[1]		
Population age group	**Number of respondents**	**Number of consumers/ percentage of respondents**	**Mean (all respondents, consumers and non-consumers)**	**50th percentile (median, consumers only)**	**90th percentile (consumers only)**
2+ years[2]	12,153	76/<1%	0.0005	0.063	0.146

[1]Molluscs included mussels, octopus, oysters, scallops, squid or calamari (ABS, 2016b); [2]Young children generally tend to eat more food per kilogram of body weight than older children or adults, and should be considered separately in dietary exposure studies. However, large amounts of bivalve molluscs are typically not consumed by this age group (0 consumers from 779 respondents in the 2-6 age category).

A standard serving of seafood for adults is 150 g/day and for children up to 6 yrs is 75g/day (FSANZ, 2011). These standard serving sizes are equivalent to approximately two dozen (150 g of shellfish tissue) and one dozen (75 g of shellfish tissue) oysters. Based on NSW aquaculture production records, there were 5,273,919 dozen Sydney rock oyster and 468,294 dozen Pacific oyster produced during the 2015/16 financial year. This is approximately 2,871,107 standard servings. If 1 % (Table 23) of the current Australian population (24,127,200 as of June 2016 (ABS, 2016a)) were consuming this product, this would equate to approximately 12 available servings of NSW shellfish per person per year. As most of the shellfish produced in NSW is sold within the state, consumption by 1 % of the NSW population (7,725,900 as of June 2016 (ABS, 2016a)) is equivalent to approximately 37 available servings per person per year. These estimates do not consider mollusc consumption from other sources and assumes that each person would consume the same amount of shellfish, which is unlikely. Frequency of consumption would also vary seasonally, peaking during Christmas and Easter holiday periods. It is more probable that consumption patterns would be lower as the daily serving size for median consumers is less than half that of high-level (90th percentile) consumers (Table 23). Consideration of this smaller serving size would more than double the available portions estimated above, however, the implementation of harvest area closures when algal and biotoxin limits are exceeded substantially reduces the likelihood of contaminated product entering the marketplace.

Risk characterization

'The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.'

It is well recognised that effective monitoring programs that implement the established regulatory limits for algal toxins can mitigate negative impacts to human health. As noted in the previous section, links to illnesses can still occur after illegal harvest from closed harvest areas (Lawrence et al., 2011). Sumner et al. (2014) provided a qualitative risk assessment based on shellfish harvest 'illegally' during a harvest area closure, or from recreational harvest in unmonitored locations (Table 23).

Since the establishment of the current system for monitoring algae and biotoxins in classified NSW shellfish aquaculture areas, there have been zero reports of human illness linked to algal bloom events. This is due to the ongoing risk management approach undertaken by the NSW Food Authority. Following the process of Sumner et al. (2014) a qualitative assessment was applied to the existing program. Based on the current monitoring approach, and the low presence of algal toxins recorded to date, the risk of human intoxication from shellfish harvest from NSW aquaculture areas is ranked as low (Table 24). There is a clear line of communication between the NSW Food Authority and NSW shellfish aquaculture producers if a closure due to an algal bloom and/or detection of toxins has been implemented. In addition, positive biotoxin results above the regulatory limit are reported to DPI Fisheries who provide notification to recreational fishers and indigenous fishing groups. Warnings of the risk fo shellfish poisoning are also issued through the appropriate media channels. The risk ranking of high for illegal harvest (Table 23) would still apply if warnings against harvesting shellfish from locations affected by algal blooms were ignored.

Table 24 Qualitative risk estimate of likelihood of shellfish poisoning occurring from recreational gathering or harvesting during a closure (from Sumner et al. (2014)).

Element	
Severity of hazard	High
Likelihood of occurrence	High*
Growth in product likely to cause disease	None
Effect of production/process on pathogen/agent	No effect
Consumer terminal inactivation step	None
Epidemiological links	Well established
Comments	Severity of the hazard, together with no reduction during processing and preparation for consumption lead to high risk rating
Risk Rating	**High**

*Note that, normally, the state would issue public health warnings. However, illnesses in Tasmania have occurred during closure events and when media coverage should have prevented recreational harvest. Accordingly, the 'high' rating is appropriate for those who ignore warnings

Table 25 Qualitative risk estimate of likelihood of shellfish poisoning occurring from a product harvested under the established algal and biotoxin monitoring program in NSW shellfish harvest areas.

Element	
Severity of hazard	High
Likelihood of occurrence	Low
Growth in product likely to cause disease	None
Effect of production/process on pathogen/agent	No effect
Consumer terminal inactivation step	None
Epidemiological links	There have been no illness reports linked to algal events in NSW shellfish harvest areas since the establishment of the current monitoring program.
Comments	Harvest area closures based on effective algal and biotoxin monitoring would remove potentially harmful product from the market and lead to a low risk ranking.
Risk Rating	**Low**

Discussion

The current testing regime of fortnightly phytoplankton plus monthly biotoxin appears to be working well with detection of bloom events and closure of harvest areas prior to toxin levels exceeding regulatory limits. The frequency of testing is commensurate with low risk harvest areas (Figure 3) (Andersen et al., 2003, Silke 2014). The current procedure for the NSW Food Authority is to continually review phytoplankton and biotoxin test results as they are reported (fortnightly and monthly). Sampling frequency is intensified upon breach of PAL triggers in seawater samples and/or detection of positive biotoxins in shellfish flesh in individual harvest areas (refer Figure 2).

The long-term phytoplankton and biotoxin monitoring dataset has been used to determine the risk of marine biotoxins for shellfish aquaculture areas in NSW. The current risk management approach is based on this risk assessment. Each harvest area is continually assessed in near-real time as results are reported from the laboratories, and any potential risks are immediately managed through increased sampling frequency and/or harvest area closures. Annual and triennial reviews for each harvest area take trends and developments with regards to potentially harmful species into consideration. For example, the recent detection of PSTs above the regulatory limit in Twofold Bay has highlighted the potential for reoccurring blooms in this location by *Alexandrium* spp., which will result in increased sampling frequencies. Conversely, there is a lack of historical evidence to demonstrate a risk to shellfish consumers from NSTs. Based on the current data, a risk management decision was made not to routinely test shellfish for NSTs. However, all phytoplankton samples are assessed for potentially NST-producing species.

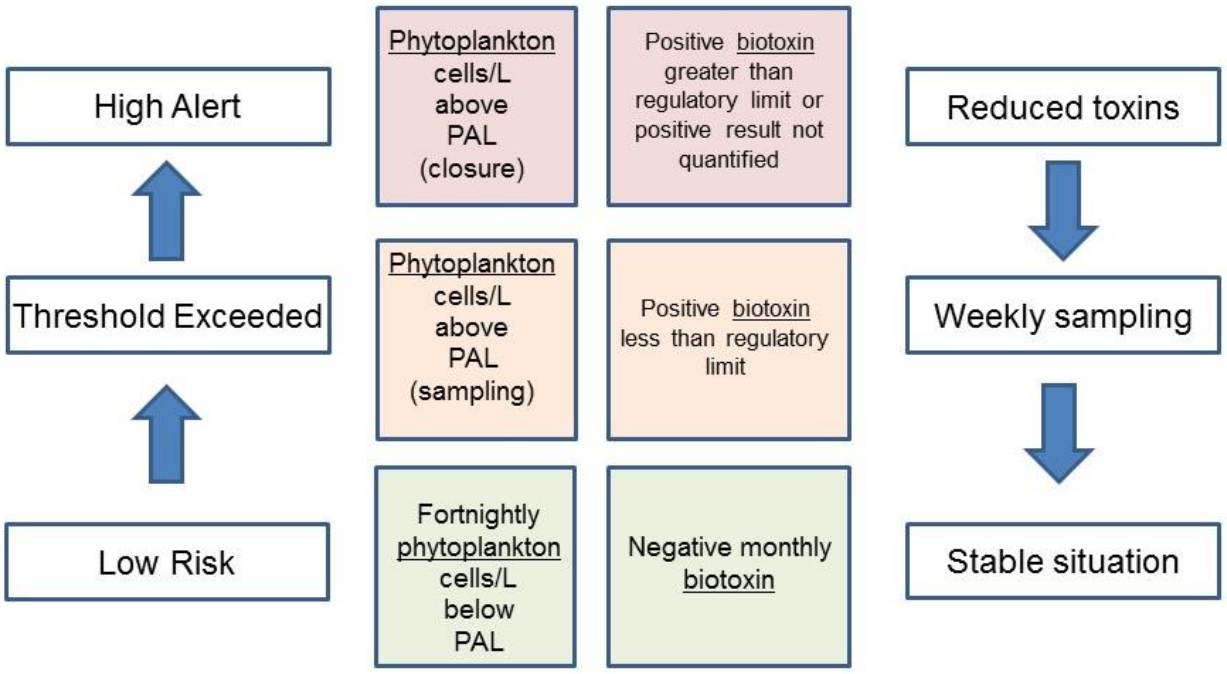

Figure 3. Operational action plan for NSW Food Authority Shellfish Harvest areas, incorporating three Silke (2014), with three monitoring modes and alert scenarios (refer Andersen et al, 2003 & Silke, 2014).

As part of this risk assessment, the timeline of phytoplankton concentrations in Wagonga Inlet were reviewed leading up to the positive, above regulatory limit, biotoxin event (2010). In this case, PAL flesh sampling or closure triggers were not reported from seawater samples before the positive (29 mg/Kg DA) was reported. *P. delicatissima* group were reported at 210,000 cells/L on 6 April 2010 and > 2.6 million cells/L on the same day that the initial positive biotoxin result was collected (20 April 2010). With respect to Wagonga Inlet, the 2010 bloom event occurred following extreme and anomalous environmental conditions. As such, the NSW Food Authority has considered it unnecessary to manage such conditions within the routine monitoring program. However, should similar atypical incidents occur in the future in Wagonga Inlet, or any other NSW aquaculture area, the frequency of routine sampling would be increased.

Regulation of routine monitoring requirements

As of July 2014, stricter guidelines regarding the timing of routine samples have been enforced. Biotoxin sampling is tracked on an on-going basis. If the required biotoxin or phytoplankton results for an open harvest area are not reported within the required timeframe the area is closed until a sample result is received. Across the state 118 closures (1 July 2014 - 31 December 2016) have been enacted for failure to collect the required samples. This has led to improved sampling compliance across all estuaries.

Findings of Ajani et al. (2013)

The study by Ajani et al. (2013) identified:

- 45 potentially harmful phytoplankton taxa in 31 oyster-growing estuaries,
- an upstream/downstream zonation within estuaries, with benthic species favouring upstream harvest areas.
- phytoplankton seasonal distribution was variable across estuaries but suggested a winter minima,
- a latitudinal diversity gradient - fewer harmful species were identified in the northern estuaries and an increasing number of species occurred southward,
- estuary modification, and to a lesser extent rainfall, revealed a significant correlation to phytoplankton abundance – there was a general trend for moderately modified estuaries, with slow flushing, to have a greater abundance of toxic species compared to those that are extremely modified or largely unmodified.
- Wagonga Inlet, Wallis Lake and Hawkesbury River were identified as the highest-risk NSW estuaries. These estuaries had the greatest number of PAL exceedances, primarily due to *Pseudo-nitzschia* spp. (Wagonga Inlet and Wallis Lake) and *Alexandrium* spp. (Hawkesbury River).

Overall, the study emphasised the continued need for regular algal and biotoxin monitoring in the oyster-growing estuaries of NSW, as well as developing a more thorough understanding of the toxicity of *Pseudo-nitzschia* spp. However, from a regulatory perspective, the results indicate that it might be possible to reduce the number of sample sites in some estuaries, due to the lower numbers of harmful taxa observed.

Conclusion

In NSW shellfish aquaculture areas, significant phytoplankton blooms have been infrequent. Harvest area closures have been primarily due to rainfall and/or salinity exceeding the trigger levels used as indicators of microbial and viral water quality. However, increasing demand on coastal resources from an increasing population (ABS, 2016a) and the potential for spatial and temporal distributions of harmful phytoplankton species to be altered dramatically by a changing climate (Hallegraeff, 2010) are key future challenges. The application of quantitative methods in routine biotoxin risk assessment enables a better-informed approach for management of shellfish harvest programs, including a faster return to harvest on some occasions, and potential public health risks.

Ongoing review of algal and biotoxin monitoring data should continue as part of the annual review process for each estuary, and it is recommended that long-term trends be reviewed each decade.

Future directions

- Previously, the NSW Food Authority has supported a project for the detection of the saxitoxin (PST)-producing gene in seawater. In addition, researchers at UTS are currently developing a genetic probe to target *P. cuspidata* (AST – producer, Wagonga Inlet) There is potential for future application of molecular applications in the routine monitoring context, particularly for species which are challenging or impossible to identify via light microscopy.
- Recently, the NSW Food Authority partnered with researchers from NSW Dept. of Primary Industries, University of Technology, Sydney and University of Sydney to determine differential accumulation of PSTs in shellfish. The results of this study suggested that during bloom events different shellfish species may be managed separately.
- Since a major PST bloom event in Tasmania during 2012 (Campbell et al., 2013), particular attention has been focused on elucidating the toxicity of different strains of *Alexandrium* spp., in collaboration with University of Technology Sydney, during occasions when cell numbers are elevated.
- Monitoring program data has been used to identify occurrences of *Gambierdiscus* spp. (potential ciguatoxin producers) and *Ostreopsis* spp. (potential palytoxin producers) for further study by researchers.

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