CAWTHRON INSTITUTE

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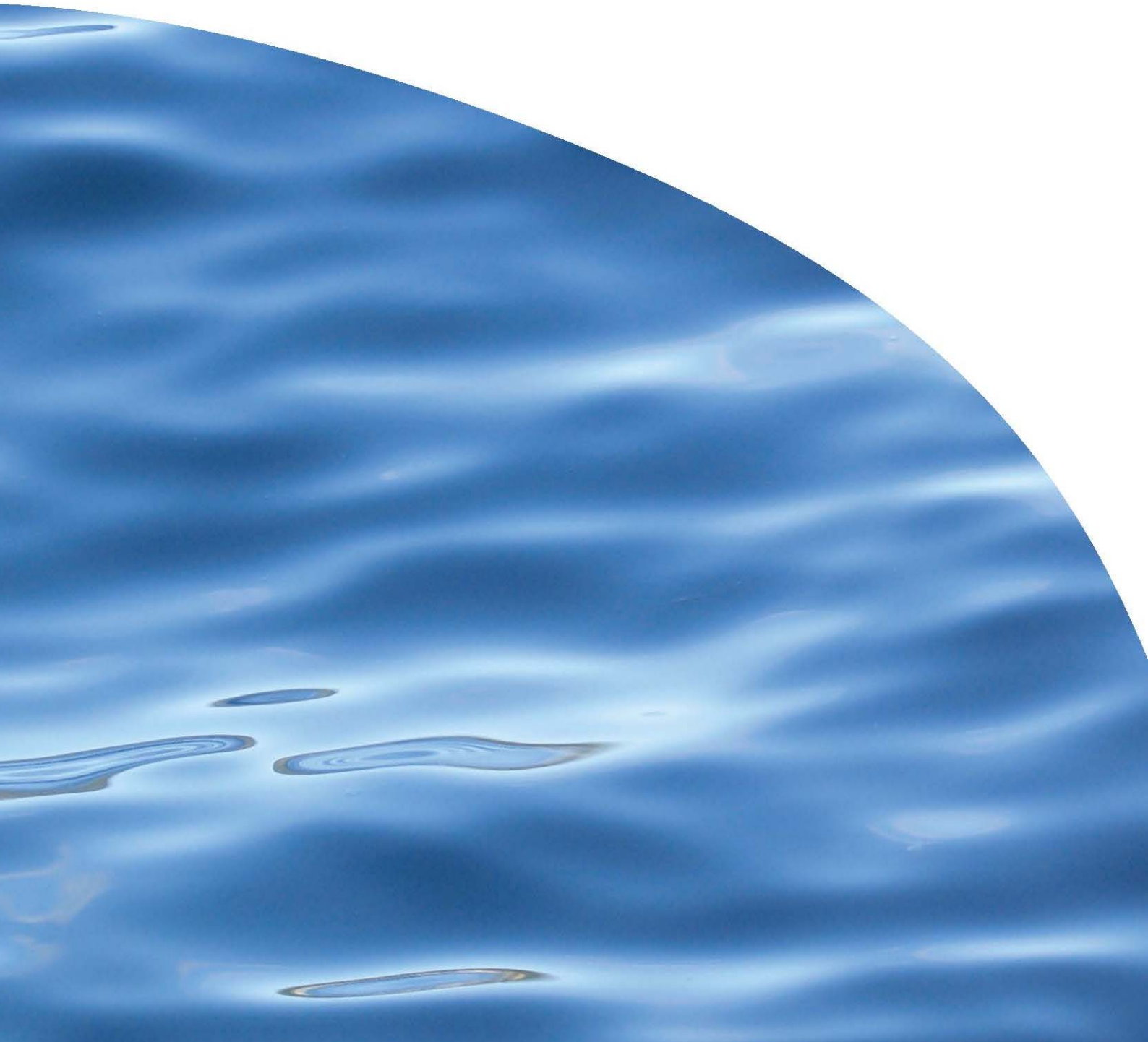
NEW ZEALAND PARALYTIC SHELLFISH POISONING UPDATE: 2014



NEW ZEALAND PARALYTIC SHELLFISH POISONING UPDATE: 2014

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Prepared for the Ministry for Primary Industries, Food Safety

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EXECUTIVE SUMMARY

- The third annual monitoring of the PSP-toxic *Alexandrium catenella* bloom in Opua Bay, Tory Channel, was carried out during the 2013–2014 summer.
- Weekly updates on the bloom progression were provided to Marlborough Shellfish Quality programme (MSQP), Aquaculture New Zealand (AQNZ), marine farmers, Ministry for Primary Industries (MPI), Marlborough District Council (MDC), Nelson Marlborough District Health Board (NMDHB), and other interested parties.
- The intensity and duration of the bloom was somewhat different than preceding years but the timing of its onset was the same, with increasing numbers of motile cells appearing in the water column during the last week of January 2014.
- A public health notice advising against the consumption of shellfish throughout Queen Charlotte Sound was in force for one month between 25 February and 31 March 2014.
- The effect of tides, winds and solar irradiation on circulation and stratification clearly determine the dynamics of the bloom in Opua Bay and the role it plays as an incubator that disperses cells to other areas of Queen Charlotte Sound.
- From late January through February high numbers of cells rapidly appeared in mid water column and near-bottom waters of the bay. A maximum was reached on the 26 February which was followed by a precipitous decline between this date and 3 March.
- The collapse of the bloom was co-incident with the spring tide and a water column mixing event following a 10-day period between 23 February and 5 March when surface waters steadily decreased in temperature by around 3°C (to < 16°C).
- It is unknown whether the very rapid bloom collapse in early March 2014 was due to cells being diluted and flushed out of the bay or whether there was a biological response such as cell death or synchronised mass encystment.
- Analysis of temperature profile data each January to April from 2012–2014 showed that the tide cycle plays an important role in water column stratification in Opua Bay and probably directly affects the dynamics of the *A. catenella* bloom in both positive and negative ways.
- Application of a statistical model showed there was a significant association between spring tides and episodes of cool bottom water intrusion into Opua Bay. When this

occurs during periods of high air temperature and solar irradiation this strengthens stratification (*i.e.* increases the potential energy anomaly) and provides a regular supply of nutrient enriched water from Tory Channel which helps sustain the bloom.

- However, in late February / early March 2014 this occurred when surface waters were cooling, probably due to wind and low air temperatures which led to water column mixing and termination of the bloom.
- Preliminary simulations of a numerical model designed to predict the dispersion of *Alexandrium catenella* cells from Opua Bay are presented. These simulations show that *A. catenella* cells can be transported from Opua Bay as far afield as East Bay and the Grove Arm within realistic time-frames.
- An experiment to test the hypothesis that prior exposure of New Zealand Greenshell™ mussel, (*Perna canaliculus*), to PSP-toxins reduces toxin uptake on subsequent exposure was unsuccessful due to the rapid and unexpected collapse of the bloom. Nevertheless useful data was obtained on the variation of toxin analogue profiles in a variety of bivalve species exposed to *A. catenella*.
- The high ratio of confirmation / screen analyses observed in tuatua (*Paphies subtriangulata)* are characteristic of this species and were consistent with high ratios observed in Bay of Plenty tuatua containing long-term, low-level toxin residues. The reason is believed to be due to poor recovery of toxins during the screen test. Further research will be focussed on improving toxin recoveries from tuatua.
- An important difference was observed in the toxin profiles in the two tuatua species *P. subtriangulata* and *P. donacina. P. donacina* contained a high proportion of low toxicity decarbamoyl congeners (dcGTX2,3; dcSTX) that only occurred in very low levels in *P. subtriangulata*. The two tuatua species are very similar and occur together in surf-clam communities. The difference in toxin profiles means it is important they do not become mixed in biotoxin monitoring samples.
- A comprehensive review on the occurrence and risk to New Zealand shellfish aquaculture from paralytic shellfish toxins has recently been published (MacKenzie, 2014). A copy of this review is included here.

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1. INTRODUCTION

Paralytic shellfish poisoning (PSP) is caused by the accumulation by filter-feeding bivalves of potent neuro-toxins produced by some planktonic micro-algae (dinoflagellates). Paralytic shellfish poisoning has caused serious illness in recreational shellfish consumers in New Zealand (Murray, 2014) and it is an important quality assurance problem for the New Zealand shellfish aquaculture industry (MacKenzie, 2014).

Since the summer of 2011 it has been recognised there is a chronic problem with the occurrence of the PSP-toxic dinoflagellate, *Alexandrium catenella* in Queen Charlotte Sound, which sometimes results in widespread contamination of shellfish (MacKenzie *et al* 2011, 2012, 2013). *Alexandrium catenella* (Mackenzie *et al*. 2004) has been a common member of the phytoplankton on the east coast of the North Island for many years but it was only first observed in Queen Charlotte Sound in 2010. *A. catenella* may be in the process of colonising the coastal waters of the northern South Island.

Alexandrium catenella is a prolific producer of resting cysts that over-winter on the sea floor and germinate to produce new blooms in subsequent years. After the 2011 bloom, surveys revealed that *A. catenella* resting cysts were widespread in the sediments of Queen Charlotte Sound, and especially high numbers were found in Opua Bay off Tory Channel (Figure 1). Subsequent studies have shown that Opua Bay is an important location where blooms are generated every year and from where cells are distributed to other areas under certain weather conditions. Opua Bay is an excellent monitoring location. From observations of bloom development in the bay, predictions can be made on the probability of the appearance of cells in other areas and the likelihood of shellfish harvest closures.

With the support of the Marlborough Shellfish Quality Programme (MSQP) and resources under Cawthron Institute's Ministry of Business Innovation and Employment (MBIE)-funded, Safe New Zealand Seafood programme (MBIE contract CAWX1317), we have focused our research over the last three years on Opua Bay. This is to enable a better understanding of the autecology of *A. catenella*, and identify key environmental and biological factors that control the development, dispersion and demise of the bloom that will assist in predicting its behaviour and mitigating it effects in the future.

2. THE 2014 OPUA BAY *ALEXANDRIUM CATENELLA* BLOOM

Sampling of the Opua Bay monitoring site by MSQP began on 11 December 2013. Each week a 15 m integrated water column tube sample and water samples at six depths (0 m, 3 m, 6 m, 9 m, 12 m, 15 m) within Opua Bay (Site 7) were collected. Sub-samples were preserved with Lugol's iodine for cell counts and a 1 litre sample was collected and subsequently filtered in the laboratory for the estimation of extracted chlorophyll-a (Chl-a) concentrations. One problem of sampling at predetermined depths can be an over- or under-estimation of real depth weighted mean phytoplankton cell numbers, because of high cell numbers in thin layers in the water column. This was the first sampling season when tube samples were taken in addition to the discrete samples, to overcome this problem.

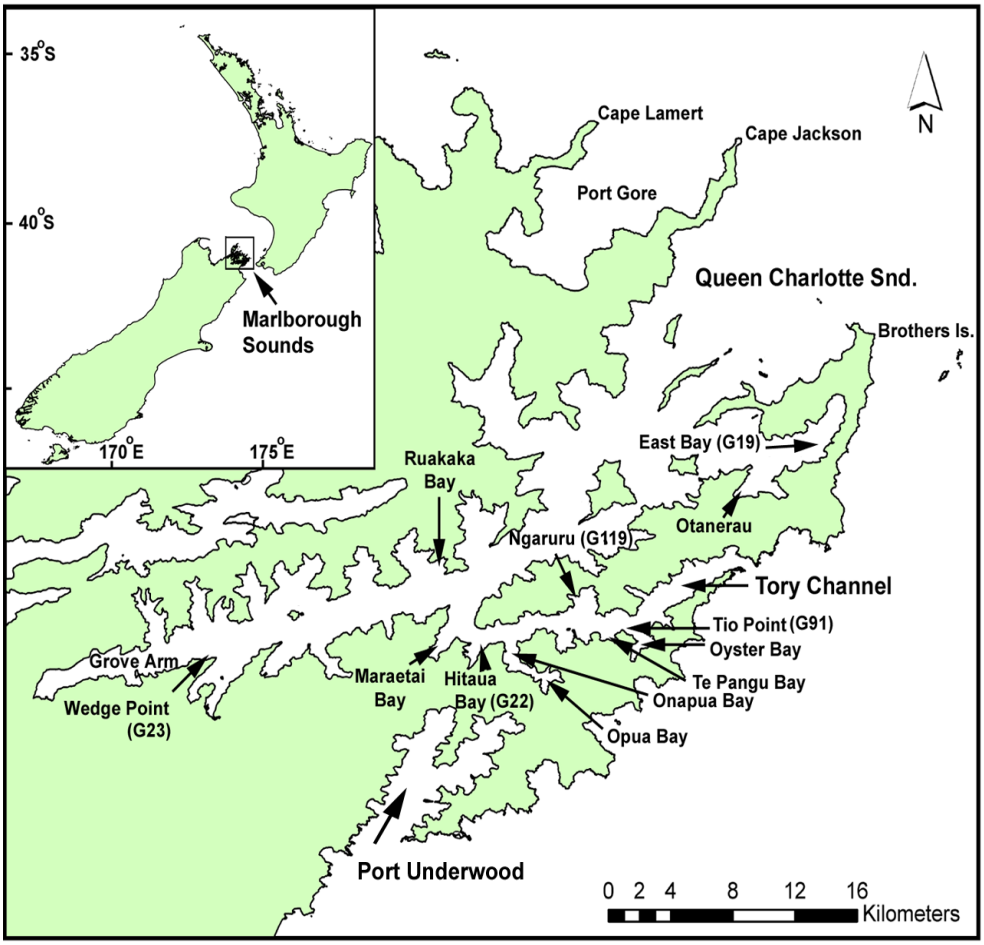

Figure 1. Locations in Queen Charlotte Sound (QCS) referred to in this report.

Low numbers of *A. catenella* cells were first observed on 23 December 2013 and began to increase rapidly from the 29 January 2014 (Figures 2 and 3). There was a large increase in cell numbers between 6 and 12 February especially in the lower part

of the water column (9 m–15 m; Figure 4). This continued until the peak of the bloom occurred during the last week of February when cell numbers reached a maximum of 466,000 cells/L at 6 m depth. Over five days between 26 February and 3 March there was a dramatic decline in cell numbers, from a mean water column concentration of 328,000 cells/L to 200 cells/L. To check that this sudden reduction in cell numbers was not due to sampling error or an increasingly patchy distribution, a tube sample survey including the 14 stations used in previous years (Figure 9), was carried out four days later on 7 March 2014. At nearly half the sites *A. catenella* was undetectable and at the others cell numbers were low (maximum 400 cells/L).

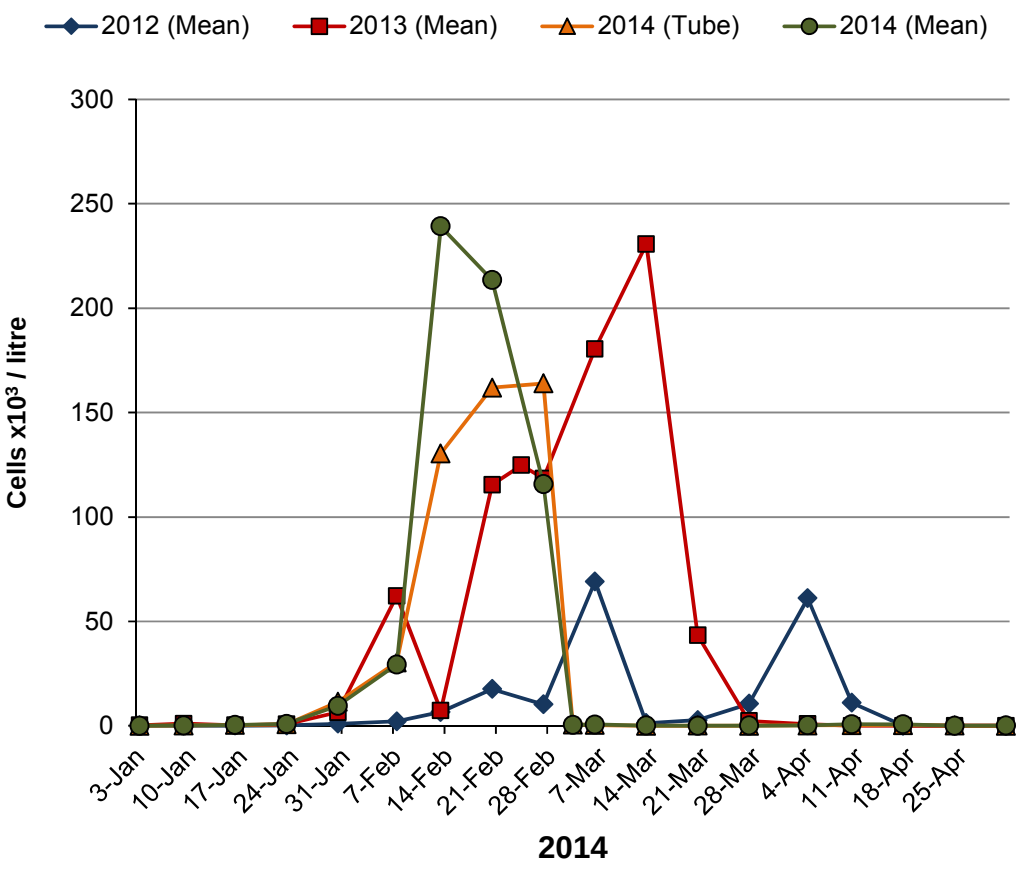

Figure 2. Mean and depth integrated (tube) *Alexandrium catenella* cell numbers in the Opua Bay water column (Site 7) over the bloom seasons each January–April from 2012–2014.

At the time, the sudden collapse of the bloom was mystifying. However subsequent retrieval of the data from the water column temperature loggers on the Opua Bay buoy (Figures 3 and 4) showed that it occurred during a 10-day period (Figure 3; 23 February–5 March) when surface waters steadily cooled by around 3°C (to <16°C) and the water column became briefly isothermal and the potential energy anomaly (PEA) approached zero at the time of the spring tide (Figure 7A). Although to some extent stratification re-developed, and a few *A. catenella* cells re-appeared, after this

episode (Figure 4), the bloom was essentially terminated. The cause of the surface water cooling over the last week of February and early March may be partially attributable to low air temperatures at this time. Air temperatures during the first week of March (1–7) at 13.2°C were 2.8°C below the long-term average (16°C) for that month (Marlborough Research Centre meteorological data). The predominance of moderate- strong north-westerly winds between 27 February and 3 March (Figure 8) may also have been instrumental in forcing cooler water from Tory Channel into the bay.

It is not known exactly what happened during the 4–5 days when the bloom crashed. It may have been that the exchange of water from Tory Channel was so vigorous that the entire population was physically flushed out of the inlet, or it may be conditions were such (*e.g.* a critical low temperature <16°C) that the cells died out or were induced to enter the sexual, resting cyst-producing stage of their life cycle. As few resting cysts were seen in water samples from the survey conducted soon after the bloom collapse, the latter possibility seems less likely. The occurrence of moderate numbers of *A. catenella* cells at other Tory Channel and adjacent sites in late February to early March suggest that cells were being exported from the inlet at this time (Table 1). It is hoped that hydrological model simulations will be able to determine whether it is possible to completely flush the inlet over this time period under these conditions.

We don't know much about the conditions under which the bloom developed and terminated in 2010 / 2011 when it first came to our attention, but we do know that high cell numbers persisted in Tory Channel and throughout the sound until early April (MacKenzie *et al.* 2011) that year. In 2012 (MacKenzie *et al*. 2012), the bloom was suppressed by high south-easterly winds in late February that lowered temperatures and directly disrupted water column stratification. In 2013 (MacKenzie *et al*. 2013) an intense bloom developed that reached a climax in mid-March under exceptionally fine, sunny conditions and rapidly declined in early April. The collapse of the bloom in 2013 was associated with a period of cooling over three days of around 2°C (to < 16 °C) and water column de-stratification during a period of neap tides (Figures 3 and 5). Bloom termination in March 2013 did not coincide with a strong wind event like that which occurred in March 2012.

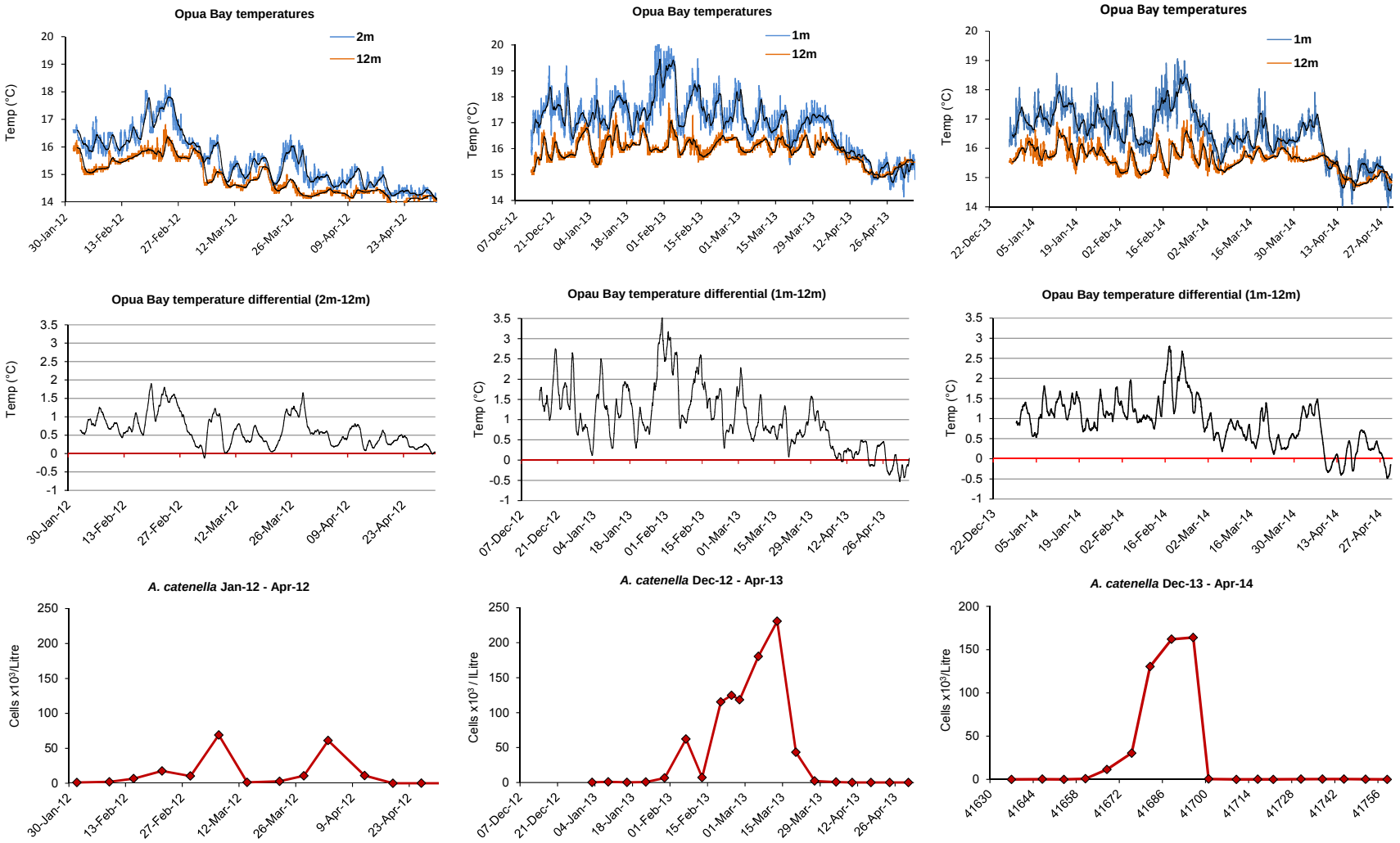

Figure 3. Water column temperatures, surface and bottom temperature differential and mean Alexandrium catenella cell numbers, each January–April from 2012–2014.

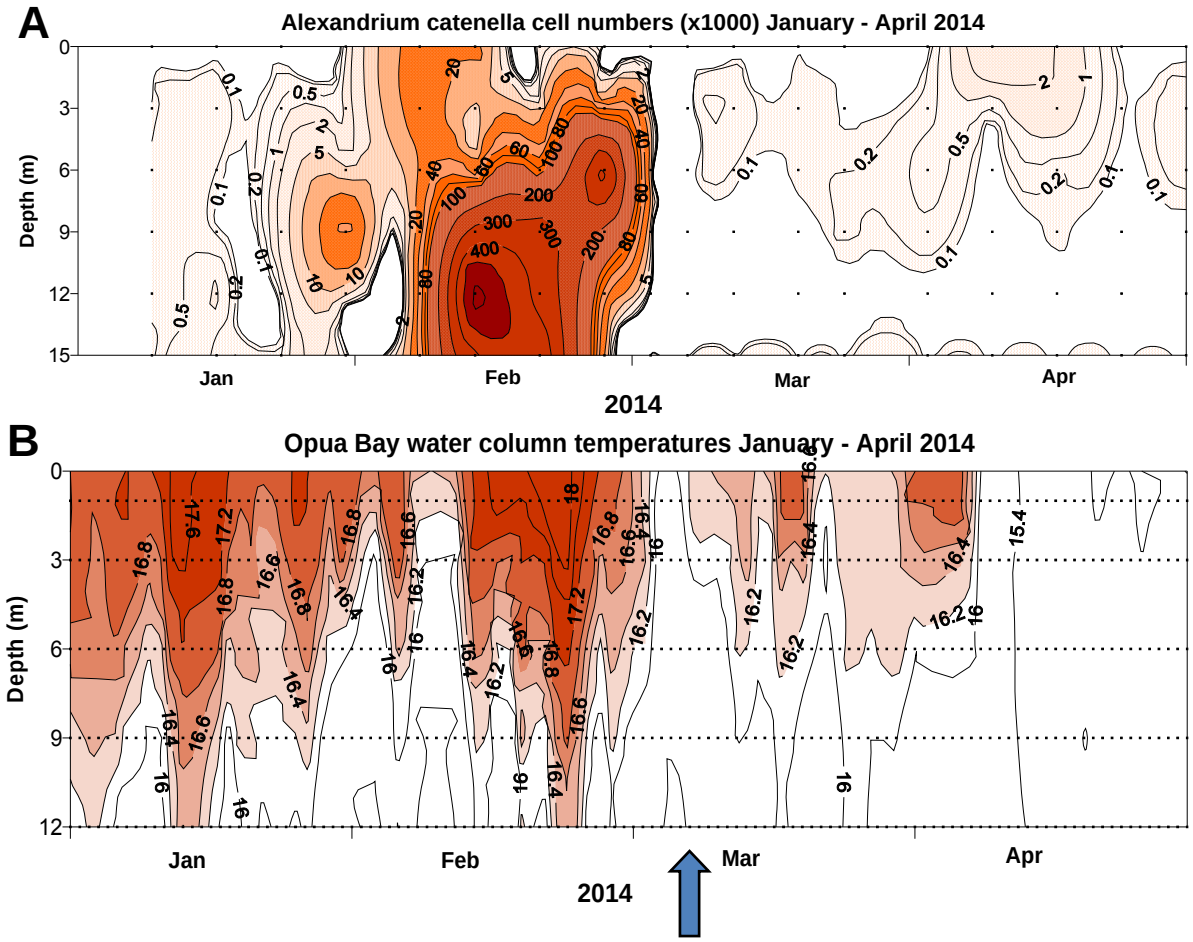

Figure 4. *Alexandrium catenella* cell numbers and water column temperature in Opua Bay January–April 2014. The arrow indicates the de-stratification episode that led to the collapse of the *A. catenella* bloom.

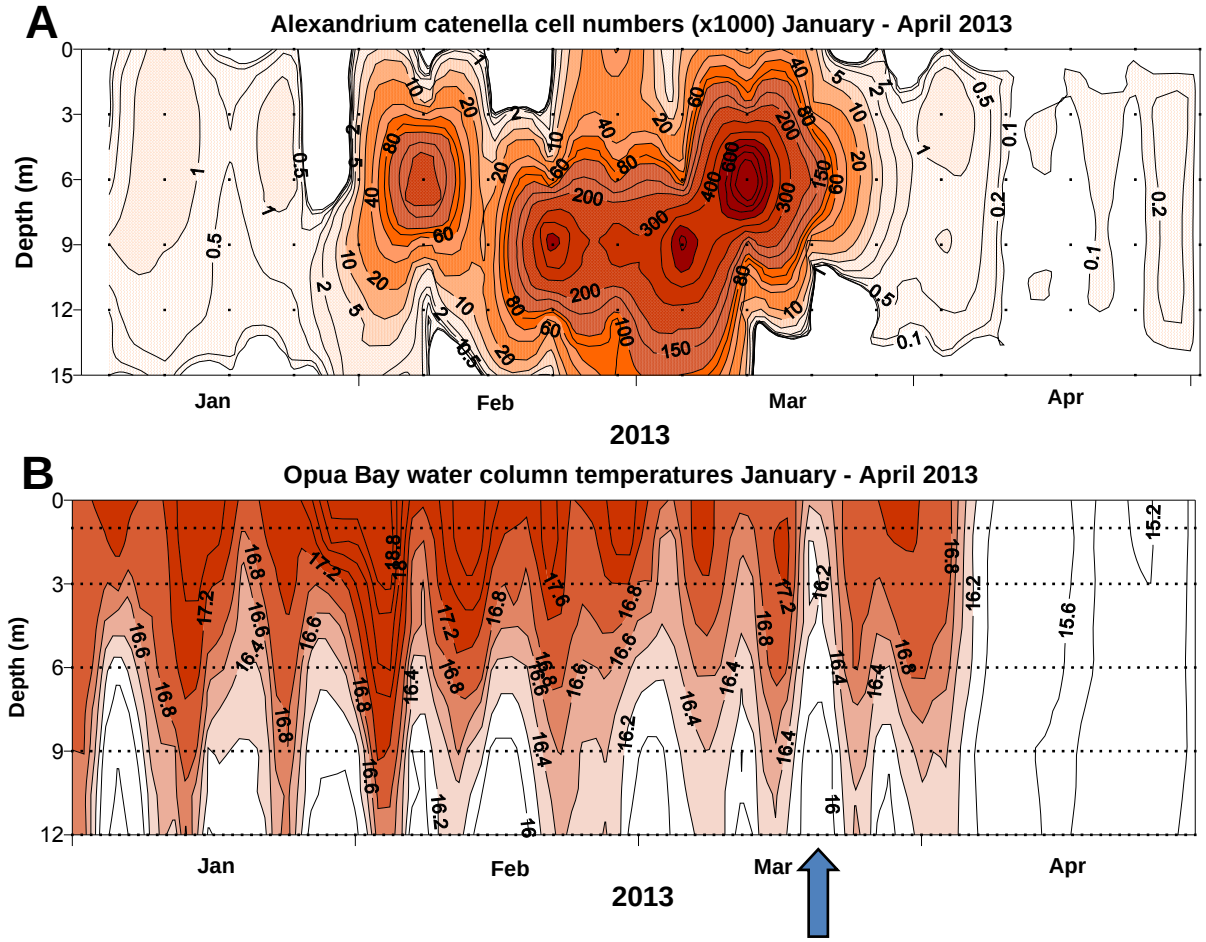

Figure 5. *Alexandrium catenella* cell numbers; A) and water column temperature stratification; B) in Opua Bay January–April 2013. The arrow indicates the de-stratification episode that led to the collapse of the *A. catenella* bloom.

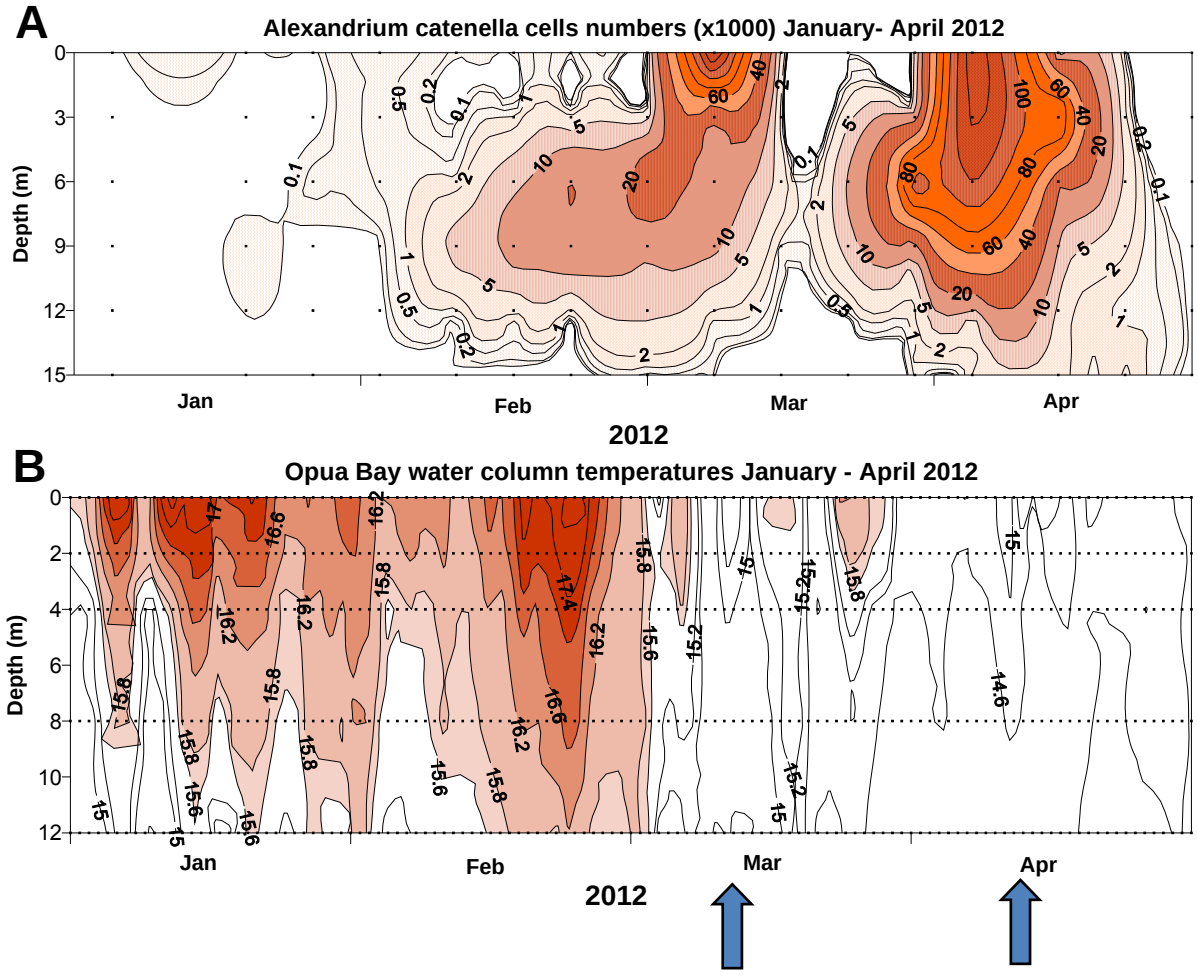

Figure 6. *Alexandrium catenella* cell numbers; A) and water column temperature stratification; B) in Opua Bay January–April 2012. The arrows indicate the de-stratification episodes that led to the collapse of the *A. catenella* bloom.

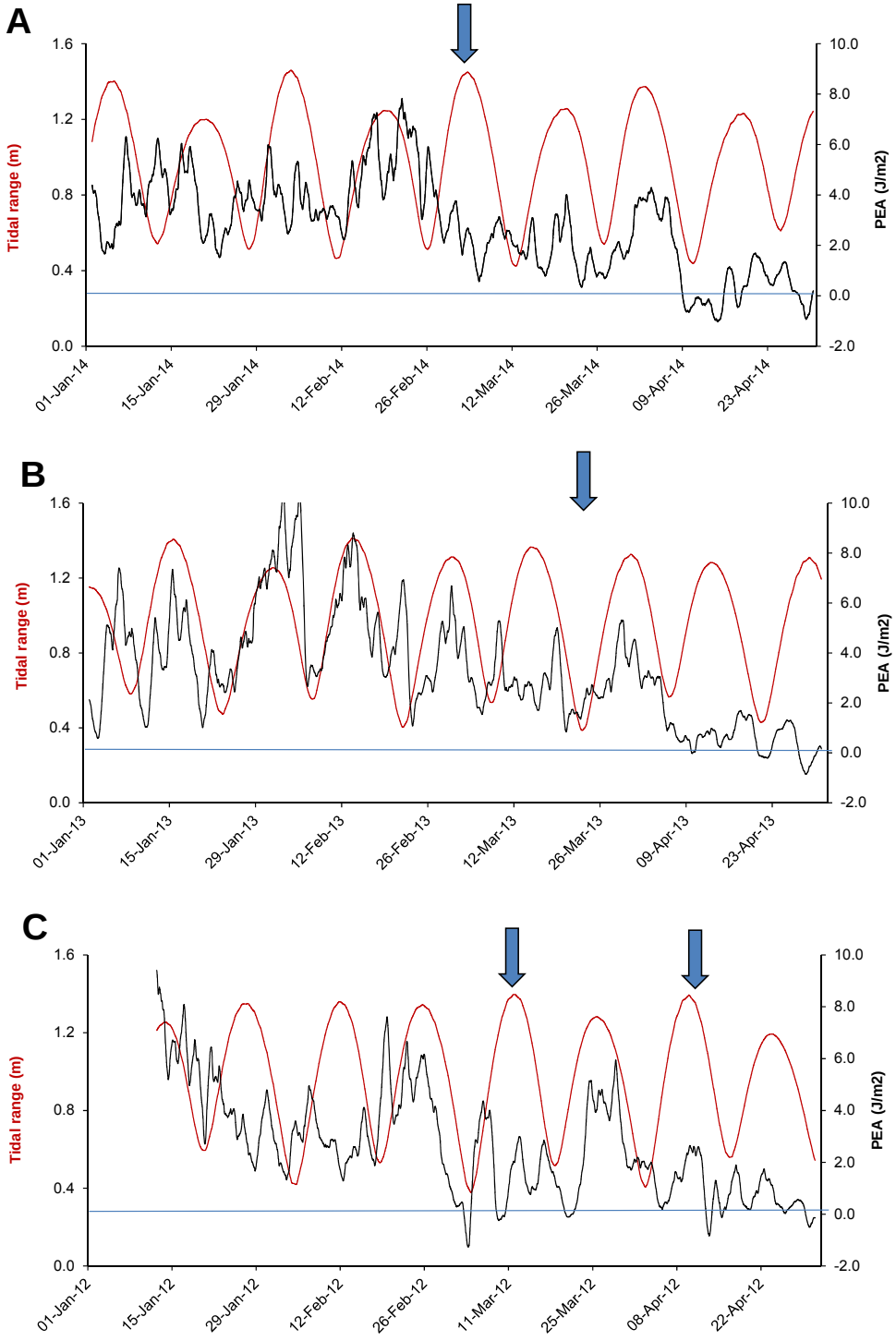

Figure 7. Tidal range (m) and potential energy anomaly (J/m^2) within the Opua Bay water column each January to April from 2012–2014. The arrows indicate the de-stratification episodes that led to the collapse of the *A. catenella* bloom each year.

2.1. Influence of wind and tide on stratification in Opua Bay

Water column stratification clearly has a major influence on the progression of the *A. catenella* bloom in Opua Bay (Figures 4–7). Comparison of water column temperature profiles (from temperature loggers on the Opua Bay raft) suggest that episodes of cold, bottom water intrusion into Opua Bay during summer thermally-stratified periods, are generally in phase with the calculated spring-maxima tidal range in Tory Channel (Figures 4–7). These regular cold water intrusions are no doubt important in refreshing the pool of inorganic nutrients (especially nitrate-N) that stimulates phytoplankton production in the bay and when surface waters are at their warmest they serve to strengthen stratification (*i.e.* increase the potential energy anomaly; PEA). Salinity generally plays a negligible role in determining water column stratification in the bay and a relatively small decrease in surface water temperature can result in complete mixing of the water column. This leads to turbulent conditions that favour diatom growth and are unfavourable to flagellates such as *Alexandrium catenella*. Strong winds can also lead to mixing and water column de-stratification and there are clearly complex interactions between wind and tide that influence water flows into and out of the bay. Preceding the bloom collapse in March 2014 winds had been light to moderate followed by persistent strong north-westerly winds between 27 February and 3 March (Figure 8), the week that there was dramatic decline in cell numbers in Opua Bay. These winds may have contributed to surface water cooling and water column mixing by forcing water from Tory Channel into the bay.

To assess what drives the establishment and breakdown of temperature stratification in Opua Bay, a statistical analysis was undertaken (Appendix 3). As expected this analysis showed that water column stratification was highly correlated with the time of year (warmer surface waters lead to stronger stratification) but it also showed that wind and tidal forcing also play significant roles.

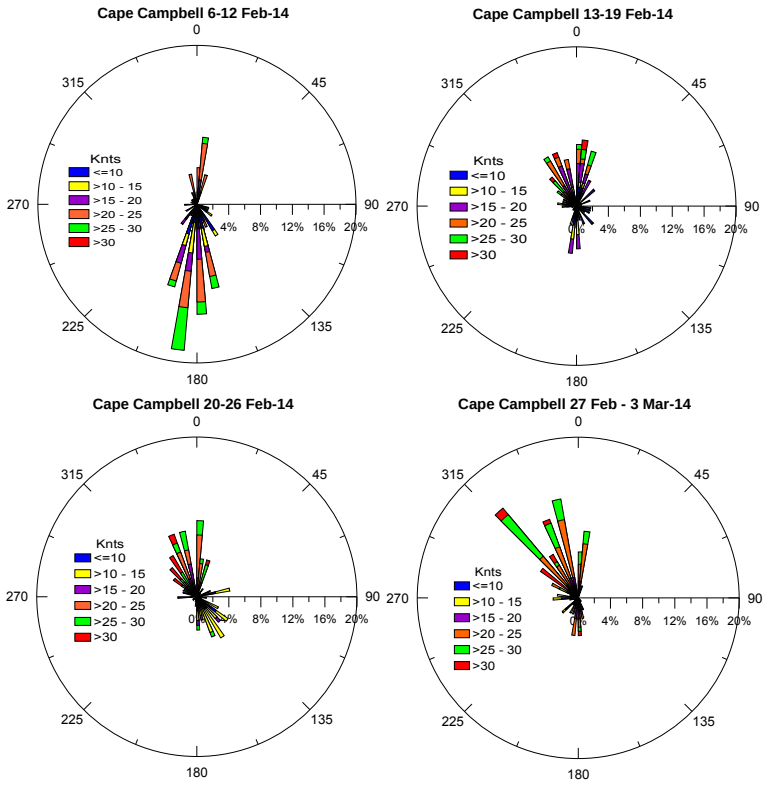

Figure 8. Wind records from Cape Campbell in the weeks preceding the water column mixing event that coincided with the collapse of the *Alexandrium catenella* bloom, 6 February–3 March 2014.

2.2. Effects on the wider Queen Charlotte Sound in 2014

The Nelson Marlborough District Health Board issued a notice advising the public against the taking of shellfish from Queen Charlotte Sound on 25 February 2014 as a result of tests on mussels collected from a jetty in Opua Bay on 19 February. The all clear to collect shellfish again was issued 33 days later on 31 March 2014.

Low to moderate numbers of *A. catenella* were observed in water samples from various monitoring sites in Tory Channel from February to April (Table 1) and there were low level PSP-toxin positive from the Hitaua Bay and Tio Point sites. There was no evidence of *A. catenella* cells or PSP-toxin contaminated shellfish from East Bay monitoring sites in 2014.

Table 1. *Alexandrium catenella* cell counts at Queen Charlotte Sound monitoring sites, February–April 2014. Data courtesy of the Marlborough Shellfish Quality Programme (MSQP) and New Zealand King Salmon Company Ltd.

QCS sampling sites	06/02/2014	11/02/2014	19/02/2014	26/02/2014	03/03/2014	12/03/2014	09/04/2014
Tio Point	-	-	500	2100	2700	100	200
Hitaua Bay	100	400	1100	1000	-	100	300
Clay Point	-	-	600	400	200	300	-
Te Pangu	-	-	600	700	200	-	-
Ruakaka	-	200	800	800	1900	200	-
Wedge Point	-	-	-	200	-	-	-
East Bay	-	-	-	-	-	-	-

dashes = not detected

3. MODELLING OF OPUA BAY BLOOM DISPERSION

Spatial modelling of Queen Charlotte Sound was undertaken to simulate the spread of *A. catenella* due to mixing and transport from a population growing at an inner Opua Bay site. A semi-implicit Eulerian-Lagrangian finite-element (SELFE) model (Zhang & Baptista 2008) was used to simulate water transport in the sound. SELFE originates from the US Center for Coastal Margin Observation and Prediction (CCMOP) and is an open-source modelling system that is ideal for carrying out high-resolution hydrodynamic simulations of coastal waters. This model is physically realistic in that it uses well-understood laws of conservation of motion and mass. Water is conserved within the model, although it can be added or removed through a single semi-circular open boundary encompassing both the north and east coasts of Queen Charlotte Sound. Water is redistributed within the model by incorporating aspects from the real-world (*e.g.* depth information, with forcing by tides and wind).

The model calculates water transport through the use of triangular volumes of varying size and is described as an unstructured finite element (FE) model. The triangular elements of the SELFE model are connected together to represent the total volume of Queen Charlotte Sound. Due to the FE approach used in the model, it is possible to resolve water properties (*e.g.* currents) at high resolution (down to approximately 20 m) within small embayments such as Opua Bay. The model has been successfully calibrated by reference to measured currents in bays in the Tory Channel region (Knight, 2012).

Modelling was undertaken by introducing passive tracers (representing *A. catenella* cells) into the water column, which are moved using transport and mixing information generated by the SELFE model. The addition of tracers was made to approximate the growth of cells within Opua Bay, so that 6,000 cells/m^2/day were added in the inner Opua Bay region throughout a modelled period of 22 days.

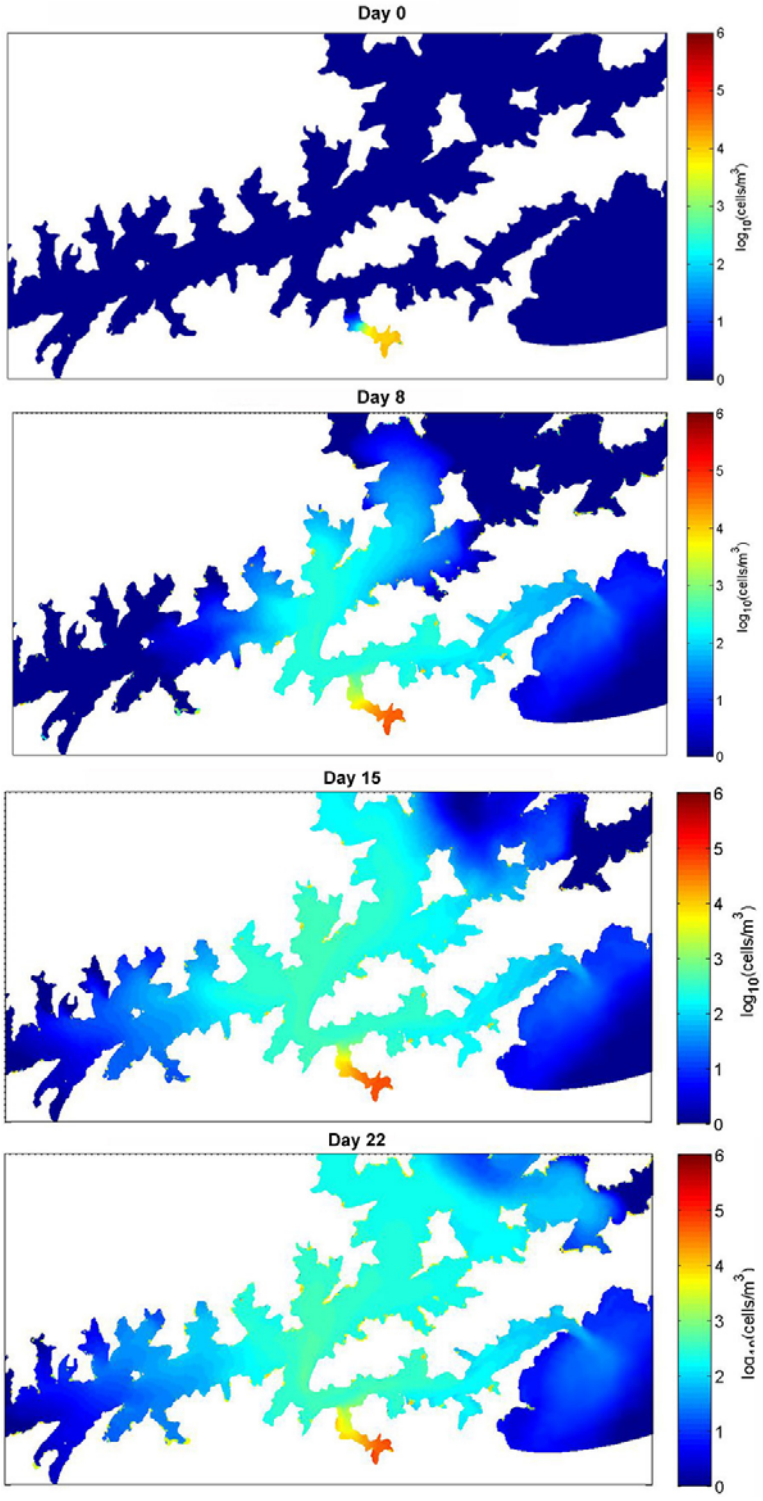

Figure 9. Simulation of daily average concentrations of *Alexandrium catenella* cells in surface waters of Queen Charlotte Sound under the influence of tidal currents over a 22-day period.

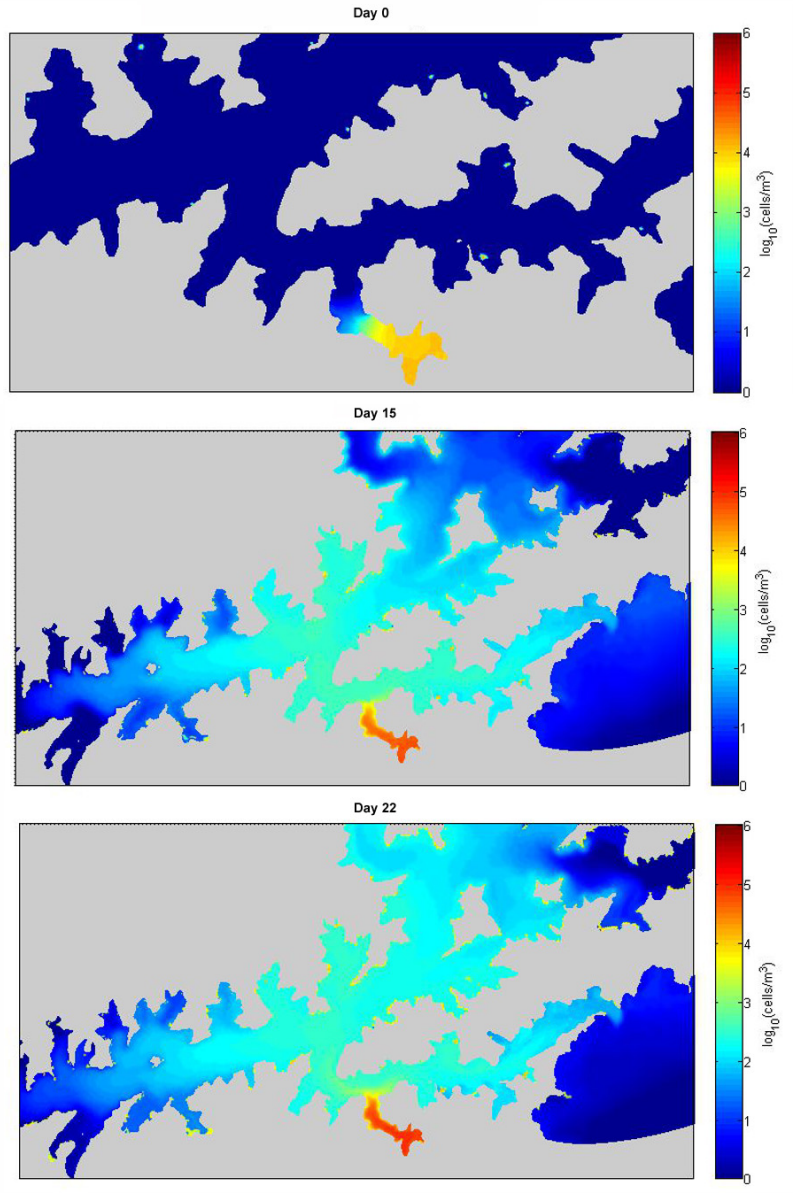

Figure 10. Simulation of daily average concentrations of *Alexandrium. catenella* cells in bottom waters of Queen Charlotte Sound under the influence of tidal currents over a 22-day period.

The results of these preliminary simulations showed that circulation within Opua Bay and the wider Tory Channel region is sufficient to mix *A. catenella* cells into surface and bottom waters of Tory Channel. After reaching Tory Channel cells can then be distributed to the inner and outer regions of Queen Charlotte Sound (Figures 9 and 10). Concentrations of cells after 22 days are highest within the region of Tory Channel surrounding Opua Bay. The model suggests these could reach daily average concentrations of about 1,000 cells/m^3 throughout the water column. Cell concentration are relatively low elsewhere in the sound. However, apart from the

continuous addition of cells in Opua Bay, there is as yet no growth function in the model that simulates the multiplication of cells as they spread throughout the sound. The model simulations show that it is feasible for cells originating from inner Opua Bay to reach as far afield as East Bay and the Grove Arm within realistic time-frames. It also shows that cells are shed from the Tory Channel entrance into Cook Strait and could conceivably travel south into Port Underwood. This model does not take into account the input of motile cells from the germination of cysts at other locations in the sound (e.g. East Bay) where cyst beds are known to exist. This model will undergo further refinement and calibration over the next year. In the future it should be possible to provide near real-time predictions of the distribution and timing of areas within the sound where trigger levels (*A. catenella* cell numbers and shellfish toxin levels) are breached based on observations in Opua Bay and other monitoring locations.

4. PHYTOPLANKTON BIOMASS AND SUCESSION IN OPUA BAY

Data on the phytoplankton biomass and species composition that has been collected over the three years in Opua Bay has revealed some consistent patterns. Routine long-term sampling of five sites throughout Queen Charlotte Sound by the Marlborough District Council and sampling carried out by the Cawthron Institute (MacKenzie *et al*. 2013) has shown that Tory Channel has persistently high levels of inorganic nutrients (especially nitrate-N) and low levels of phytoplankton biomass. This is due to the inflow of deeply mixed waters from Cook Strait, the short residence time of water in the channel and the strong currents which create a well-mixed water column dominated by diatoms at all times.

Within the Onapua / Opua inlet, the longer water residence time, weak currents and the propensity for the water column to thermally stratify leads to the development of high biomass (Chl-a) phytoplankton communities (Figure 12) frequently dominated by flagellates (Figure 13).

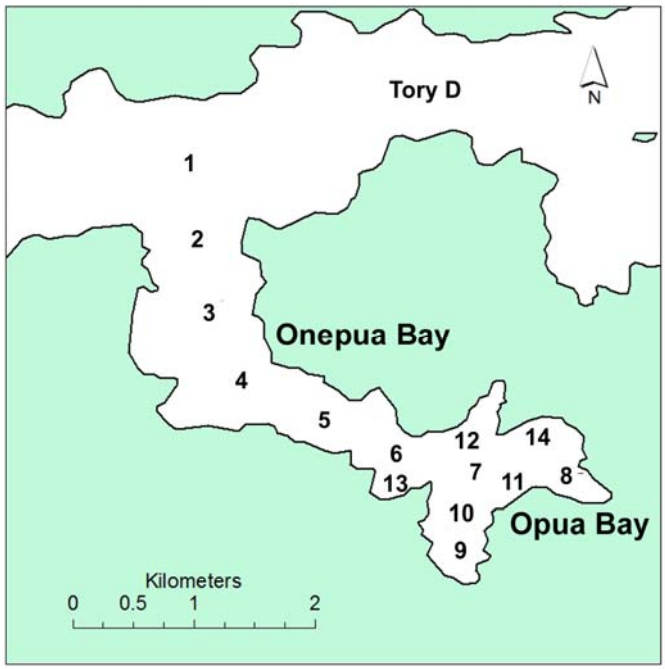

Figure 11. Selected chlorophyll-a (Chl-a) sampling sites in Tory Channel and the Onapua / Opua inlet.

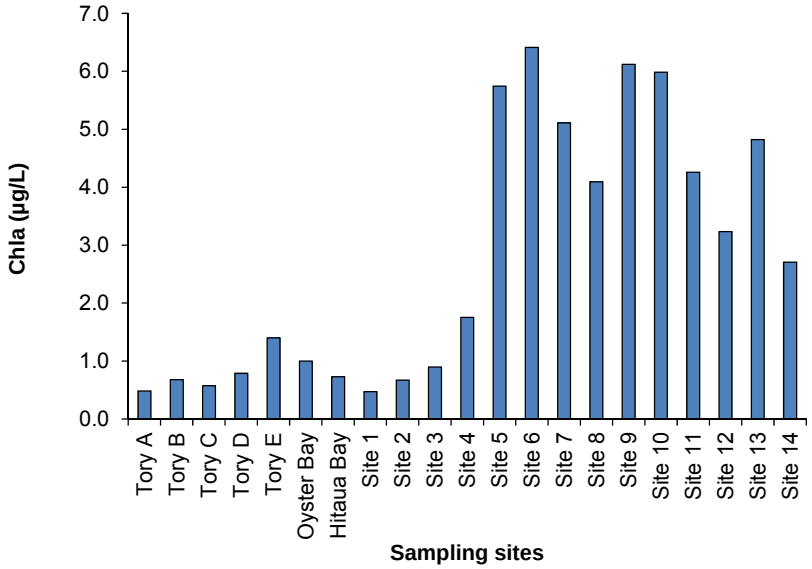

Figure 12. Concentrations of chlorophyll-a (Chl-a µg/L) in 15 metre depth integrated tube samples collected along transects in Tory Channel and the Onapua / Opua inlet, 13 March 2013.

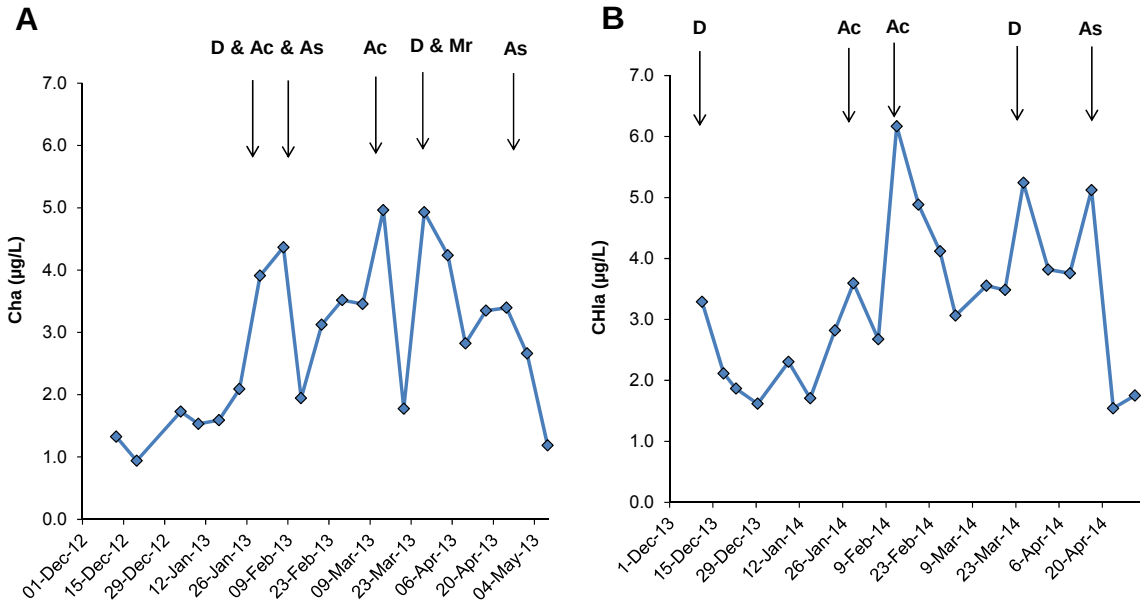

Figure 13. Mean concentrations of chlorophyll-a (Chl-a µg/L) in the Opua Bay water column at Site 7 over the summers of 2012–13 and 2013–14. The arrows and annotations indicate the dominant species or groups of phytoplankton that were dominant during the biomass peaks: *Alexandrium catenella* (Ac); *Akashiwo sanguinea* (As); diatoms (D); *Mesodinium rubrum* (Mr).

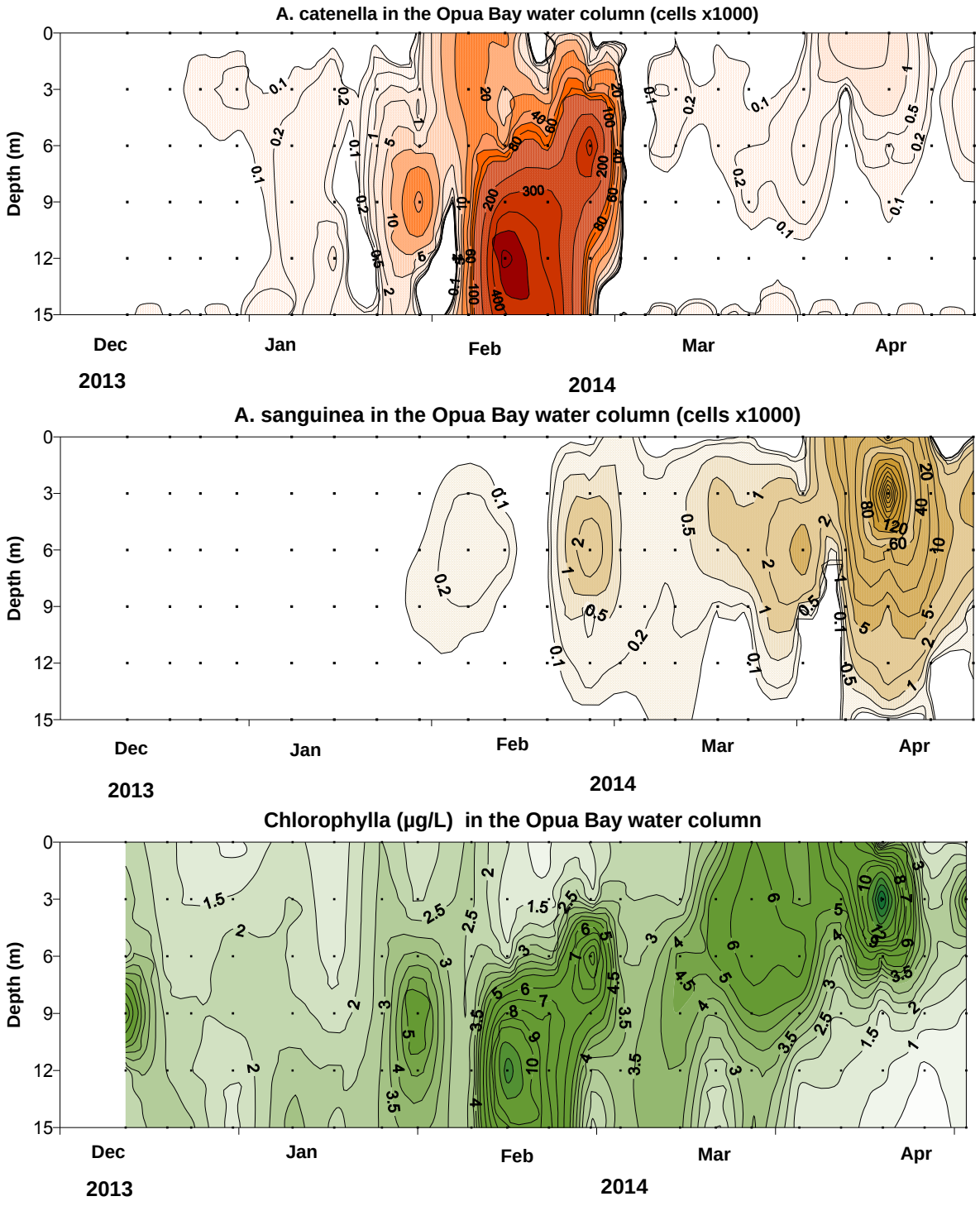

Figure 14. Distribution of *Alexandrium catenella, Akashiwo sanguinea* and chlorophyll-a (Chl-a) in the Opua Bay water column; December 2013–April 2014.

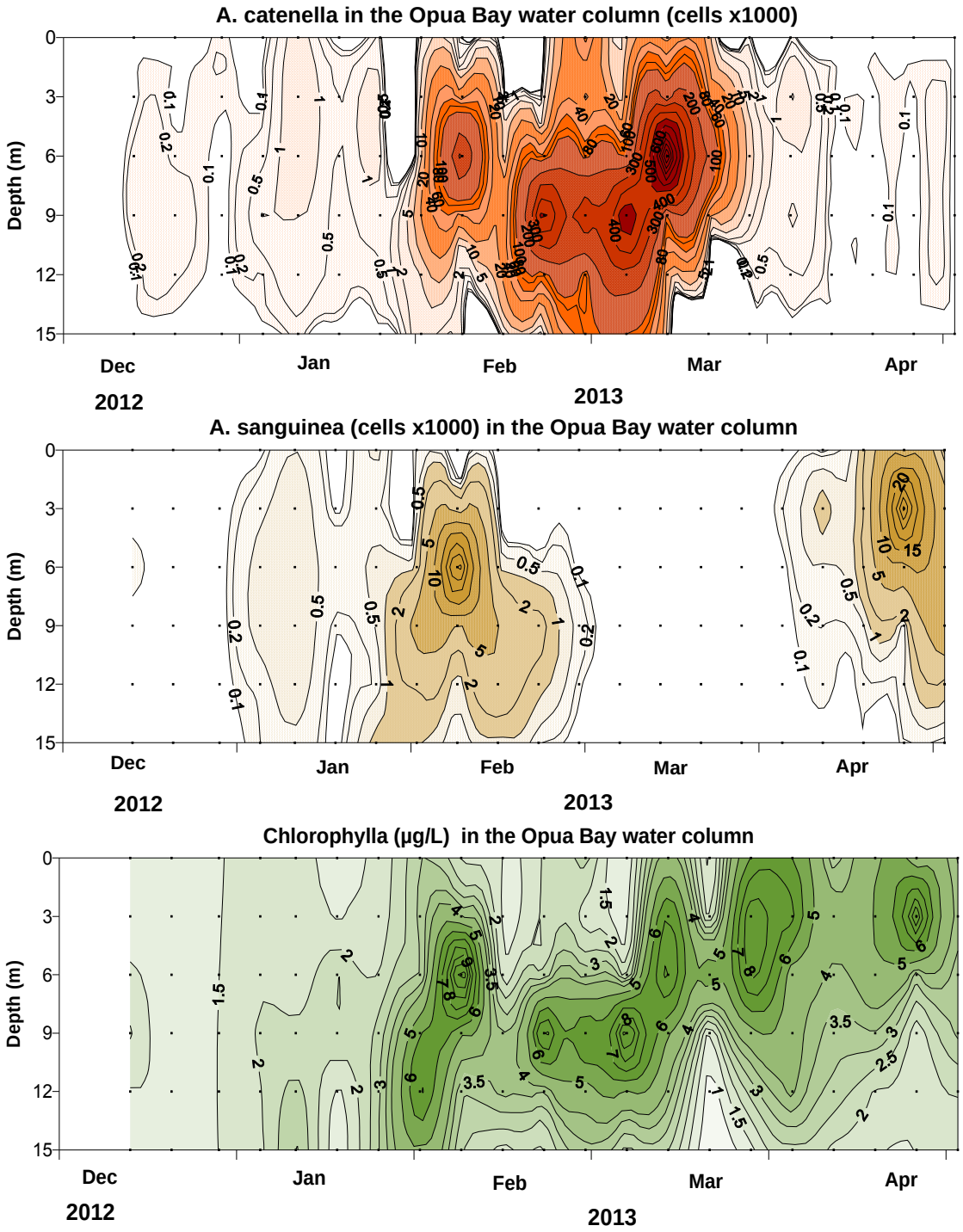

Figure 15. Distribution of *Alexandrium catenella, Akashiwo sanguinea* and chlorophyll-a (Chl-a) in the Opua Bay water column; December 2012–April 2013.

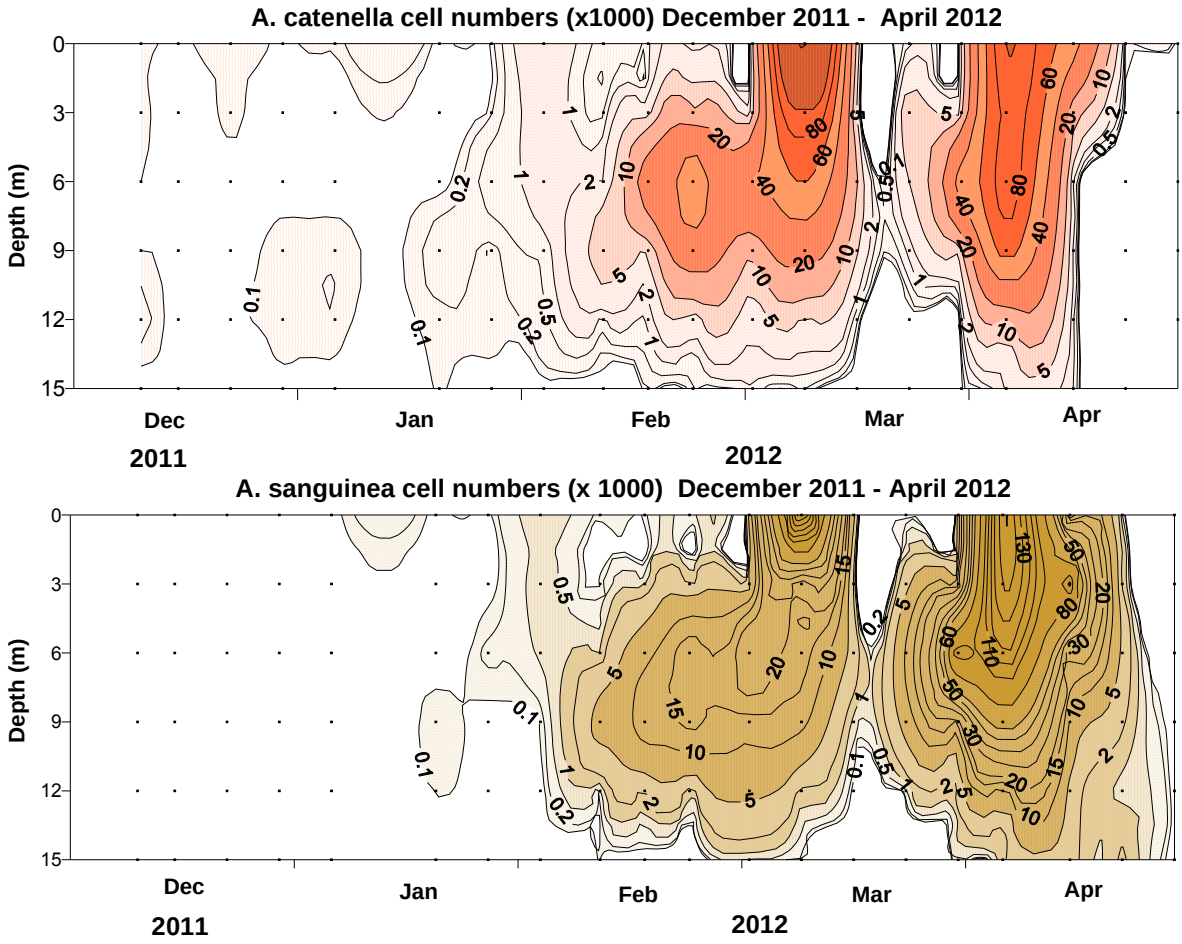

Figure 16. Distribution of *Alexandrium catenella* and *Akashiwo sanguinea* in the Opua Bay water column; December 2011–April 2012.

High numbers of *Alexandrium catenella* often dominate the summer peaks in Chl-a in January–March. Exceptionally high concentrations of Chl-a (> 10–20 µg/L) can occur at discrete depths associated with high cell densities and water column averages above 5 µg/L are common (Figures 13–15). The non-toxic planktonic dinoflagellate *Akashiwo sanguinea* often accompanies *A. catenella* early in the bloom development. It may be out-competed at the peak of the bloom, then return later in the sequence after *A. catenella* has crashed. The species *A. sanguinea* is also responsible for very high Chl-a concentrations at some times. After a de-stratification episode that leads to the demise of the *A. catenella* bloom, diatoms tend to become become dominant, sometimes accompanied by the photosynthetic ciliate, *Mesodinium rubrum* (Figure 9).

5. PARALYTIC SHELLFISH POISONING-TOXIN CONTAMINATION EXPERIMENTS

In mid-February 2014 an experiment was set up to test the hypothesis that prior exposure of the New Zealand Greenshell™ mussel (*Perna canaliculus*) to PSP-toxins under natural conditions reduced the assimilation of toxins on subsequent exposure. A previous observation (Harwood *et al*. 2013) had suggested this might occur and potentially provide the means by which juvenile mussels could be induced to acquire some resistance to toxin accumulation. Replicate batches of mussels were successfully contaminated to a high level and subsamples transferred to a toxin-free area (East Bay) to depurate prior to re-exposure. Unfortunately during the experimental depuration period the bloom unexpectedly crashed and the re-exposure phase of the experiment was not possible. Alongside *P. canaliculus,* specimens of blue mussel (*Mytilus galloprovincialis*) and two species of surf clams / tuatua (*Paphies subtriangulata* and *Paphies donacina*) were also exposed to the bloom. The surf clams were placed in baskets and in sand trays (which permitted burrowing) to test whether this had any bearing on toxin uptake. Analysis of toxin profiles provided interesting and useful data on differences in the magnitude of toxin assimilation, the metabolism of toxin residues and variations in the estimates of total toxicity by screen and confirmation analyses (Table 2).

Within one week of exposure to the *A. catenella* bloom (cell numbers up to 4.7 $\times 10^5$ cells/L) all bivalve species acquired high levels of toxicity. The saxitoxin congener profiles of *P. canaliculus* and *M. galloprovincialis* (Figure 17) were essentially the same and the relative abundance of the various analogues was consistent with profiles observed during previous contamination experiments in Opua Bay (MacKenzie *et al*. 2012, Figure 10; Harwood *et al*. 2013). Reflecting the toxin profiles in the dinoflagellate, the bivalve profiles were dominated by the low toxicity analogues C1,2 and GTX5 (B1), whereas most of the calculated toxicity was contributed by GTX1,4 and low- to trace-levels of other higher toxicity congeners. The ratio of calculated total toxicity between confirmation and screen tests (~0.5; Table 2) was slightly higher than that observed by Harwood *et al* (2013). In their study, with much larger samples sizes, mean ratios of 0.35–0.42 were obtained. After a 2-week depuration period the confirmed total toxicity of *P. canaliculus* declined by 96% (5.9–0.2 mg/kg STX equivalents) and the congener profiles became dominated by GTX5 and a low level, but higher proportion, of STX. This made a substantial contribution to the total toxicity (Fig. 18). All the N-hydroxylated STX analogues (C3,4; GTX1,4; neoSTX) had disappeared by the end of the depuration period.

When comparing the tuatua species, *P. subtriangulata* lost significantly less of its toxin load after two weeks depuration (59%) and the ratio between screen and confirmation toxicities was substantially higher (0.71–0.93) than in *P. canaliculus*. The N-hydroxylated analogues remained after the depuration period and the proportion of STX increased and became the major congener contributing to the total toxicity

(Figure 19). High confirmation / screen ratios (sometimes > 1) have been observed in samples containing low level toxin residues dominated by STX in *P. subtriangulata* from the Bay of Plenty. The reason is believed to be due to poor recovery of STX related to interference during the periodate oxidation procedure in the screen analysis. This issue is discussed in more detail in Section 6.

Table 2. Contamination and depuration of various bivalve species exposed to the Opua Bay *Alexandrium catenella* bloom.

Species	Treatment	Screen	Confirmation	Confirm / screen
		mg/kg STX equivalents		
P. canaliculus	Control sample from East Bay	nd	not tested	-
P. canaliculus	Transferred from East Bay to Opua Bay	11.0	5.8	0.53
P. canaliculus	Depurated in Opua Bay for two weeks after bloom collapse	0.4	0.2	0.5
M. galloprovincilais	Transferred from East Bay into Opua Bay	8.9	3.5	0.33
P. subtriangulata	Transferred from Rabbit Island to Opua Bay (basket)	4.4	4.1	0.93
P. subtriangulata	Depurated in Opua Bay for two weeks after bloom collapse (basket)	2.4	1.7	0.71
P. subtriangulata	Transferred from Rabbit Island to Opua Bay (sand tray)	3.3	not tested	-
P. donacina	Transferred from Rabbit Island to Opua Bay (basket)	9.2	4.8	0.52
P. donacina	Transferred from Rabbit Island to Opua Bay (sand tray)	9.4	not tested	-

nd = not detected

There was an important difference between the STX congener profiles of the two tuatua species after contamination with *A. catenella* (Figure 20). This was the appearance of a high proportion of decarbamoyl congeners, especially dcGTX 2,3 and dcSTX , in *P. donacina* but no trace of these in *P. subtriangulata.* Decarbamoyl toxins have a relatively low specific toxicity and this may be the reason why the confirmation / screen ratio for *P. donacina* was significantly lower (0.52) than for *P. subtriangulata* (0.93). The dcSTX presumably originates from the enzymatic decarbamoylation of GTX5 and the dc GTX2,3 from C1,2. Enzymes responsible for these transformations have been purified and characterised from two Japanese bivalves (Lin *et al*. 2004; Cho *et al*. 2008). *Perna subtriangulata* and *P. donacina* occur together in surf clam communities on sandy beaches around New Zealand and they can be difficult to distinguish when they are of a similar size though *P. donacina*

is usually larger. Because of the significant differences in toxin profiles it is clearly important that the two species do not become mixed in biotoxin monitoring samples.

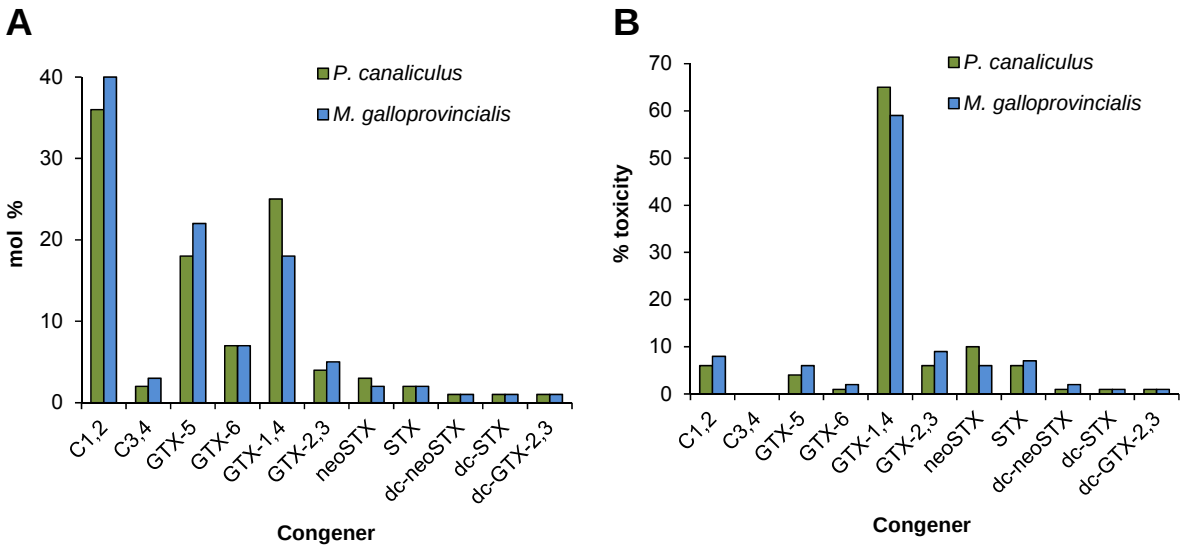

Figure 17. Saxitoxin congener profiles in green-lipped mussels (*Perna canaliculus*) and blue mussels (*Mytilus galloprovincialis*) after one week exposure to the *Alexandrium catenella* bloom in Opua Bay. The confirmed total toxicity in *P. canaliculus* and *M. galloprovincialis* was **5.8 and 3.5 mg/kg STX equivalents** respectively.

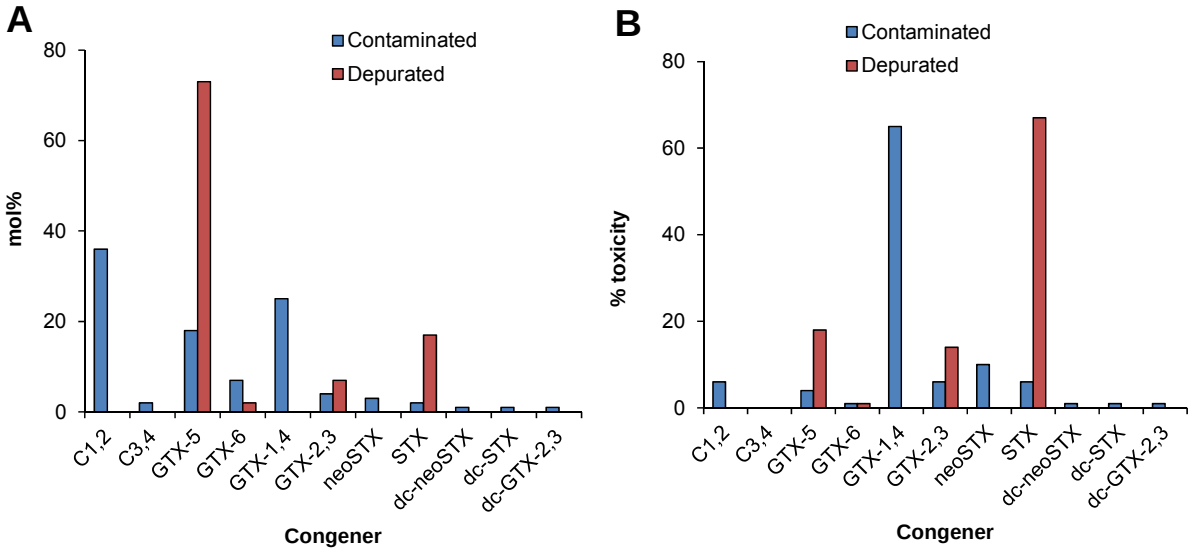

Figure 18. Saxitoxin congener profiles in green-lipped mussels (*Perna canaliculus*) after one week exposure to the *Alexandrium catenella* bloom ('Contaminated') in Opua Bay (**5.8 mg/kg STX equivalents**) and after a 2-week clearance period ('Depurated') following the collapse of the bloom (**0.2 mg/kg STX equivalents**).

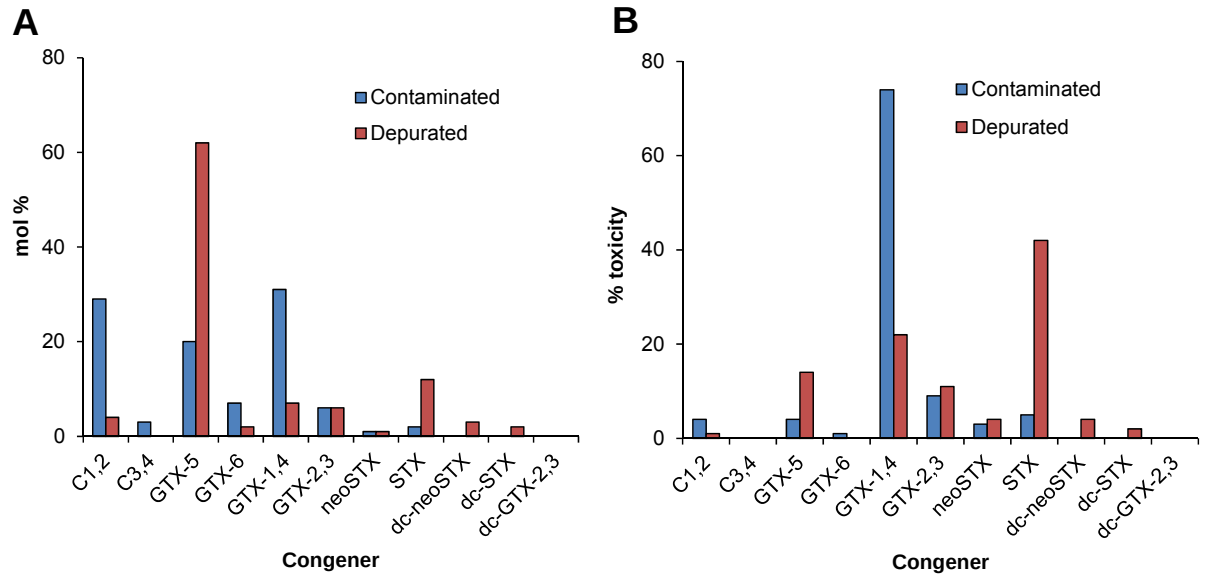

Figure 19. Saxitoxin congener profiles in tuatua (*Pahies subtriangulata*) after 1-week exposure ('Contaminated') to the *Alexandrium catenella* bloom in Opua Bay (**4.1 mg/kg STX equivalents**) and after a 2-week clearance period ('Depurated') following the collapse of the bloom (**1.7 mg/kg STX equivalents**).

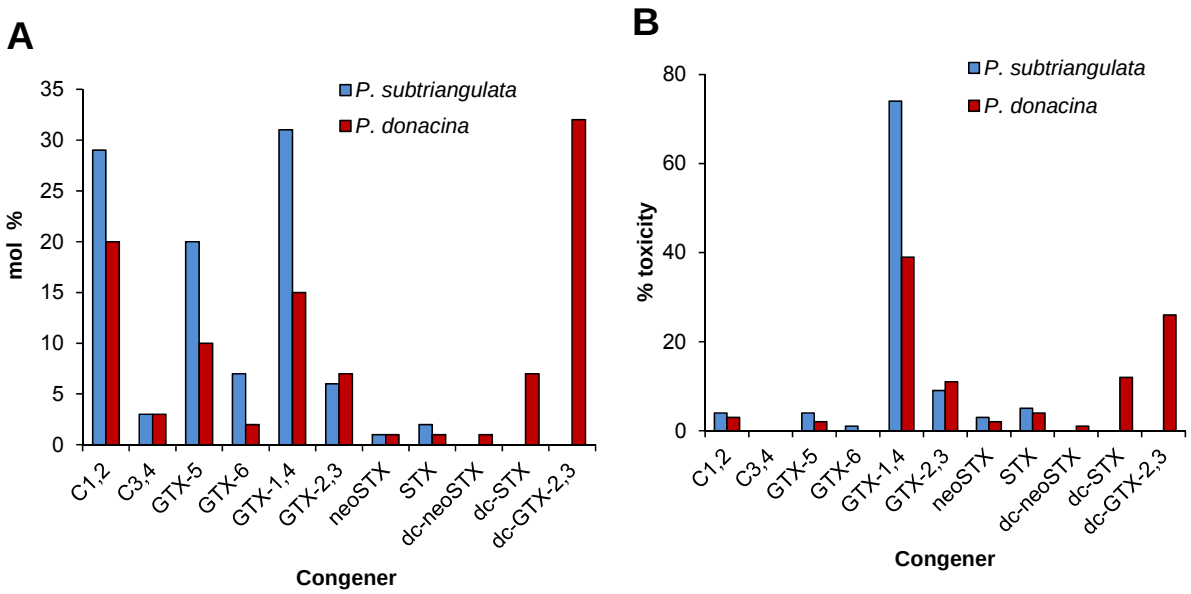

Figure 20. Saxitoxin congener profiles in two species of tuatua (*Pahies subtriangulata* and *Paphies donacina*) after 1-week exposure to the *Alexandrium catenella* bloom in Opua Bay. The confirmed total toxicity in *P. subtriangul*ata and *P. donacina* was **4.1 and 4.8 mg/kg STX equivalents** respectively.

6. INVESTIGATION OF PARALYTIC SHELLFISH POISONING CONFIRMATION / SCREEN RATIOS IN BAY OF PLENTY TUATUA

In November 2013 Cawthron began the routine testing of marine biotoxin monitoring samples from public health monitoring sites around the country on behalf of MPI-Food Safety. Within the monitoring programme there is a special focus on the Bay of Plenty beaches because of the high frequency of PSP-toxin contamination of shellfish in the area, the long term retention of toxin residues in surf clams and past human poisoning incidents (Murray, 2014). There was no new PSP contamination event over the 2013 / 2014 summer, although toxin residue concentrations over the permitted level persisted in tuatua (*Paphies subtriangulata*) throughout this period (Appendix 1). In addition to the routine monitoring samples, in February 2014 samples of *P. subtriangulata* from four sites on Papamoa Beach were collected to monitor for hydrocarbon residues from the MV *Rena* wreck, and underwent PSP screen testing (Appendix 2). These are believed to be the first analyses carried out on replicate samples from this important monitoring location.

During the period that Cawthron has been carrying out analyses on Bay of Plenty tuatua, it has become apparent that there is a technical issue regarding the relationship between screen and confirmation tests using the current method. Cawthron method 40.120 measures total PSP-toxicity of shellfish samples via a simple screen approach (Harwood, 2013). When required, a more complex confirmation procedure is used that follows the inter-laboratory tested method AOAC 2005.06. The screen utilises the universal oxidant, periodate, whereas the confirmation test uses both periodate and peroxide, with the latter being selective for non-*N*-hydroxylated compounds (STX; GTX2,3; GTX5, C1,2) only. Due to the conservative assignment of peaks in the screen test it is expected that the screen result will always be higher than the confirmation result on the same sample. However, in some cases tuatua (*Paphies subtriangulata*) samples from Papamoa (SD025), and other locations around the Bay of Plenty (n = 1 Waihi Beach SD017; n = 2 Pukehina Beach, SD028; n = 1 Ohope Beach, SD037), were found to give lower screen results (Table 3). These results were investigated further.

Table 3. Results for paralytic shellfish poisoning (PSP) screen and confirmation testing from Papamoa Beach (SD025) tuatua samples taken from November 2013–June 2014. Results are reported in mg STX equivalent 2HCl/kg.

Date sampled	Sample ID	Screen	Confirmation	Conf/Screen ratio	PSP estimate
03/11/2013	T25981-2	1.9	0.96	0.5	
17/11/2013	T26477-4	2	1.4	0.7	
01/12/2013	T27244-4	2.3	1.3	0.6	
16/12/2013	T27999-3	1.7			~1.0†
30/12/2013	T28527-6	0.96			~0.6†
12/01/2014	T28935-1	1.4			~0.8†
27/01/2014*	T29504-7	1.2	1.4	1.2	
10/02/2014*	T30202-7	0.66	1.4	2.1	
24/02/2014*	T30729-4	1.3	1.1	0.8	
10/03/2014*	T31542-8	0.59	1	1.7	
23/03/2014*	T32170-7	1.8	0.94	0.5	
08/04/2014*	T33054-7	0.81	0.97	1.2	
22/04/2014*	T33659-3	0.56	0.7	1.3	
07/05/2014*	T34274-8	1.4	1.2	0.9	
18/05/2014	T34987-3	0.56			
04/06/2014*	T35527-8	0.46	0.5	1.1	

†Estimated PSP conf values based on a calculated mean conf/screen ratio of 0.6 from analyses on 03/11/2013, 17/11/2013 and 01/12/2013.
*As a precautionary measure only confirmation results were reported for all screen positive tuatua samples after 27/01/14.

Screen results lower than confirmation results are more likely when just one highly toxic PSP-toxin analogue is present in the sample, particularly saxitoxin (STX), which has the highest relative toxicity and is quantified in the screen test from a single late eluting peak. As expected, the tuatua toxin profile that gave ratios > 1 was dominated by STX (Figure 21). To explore this observation further the difference in STX results from screen (periodate) and confirmation (peroxide) testing was investigated.

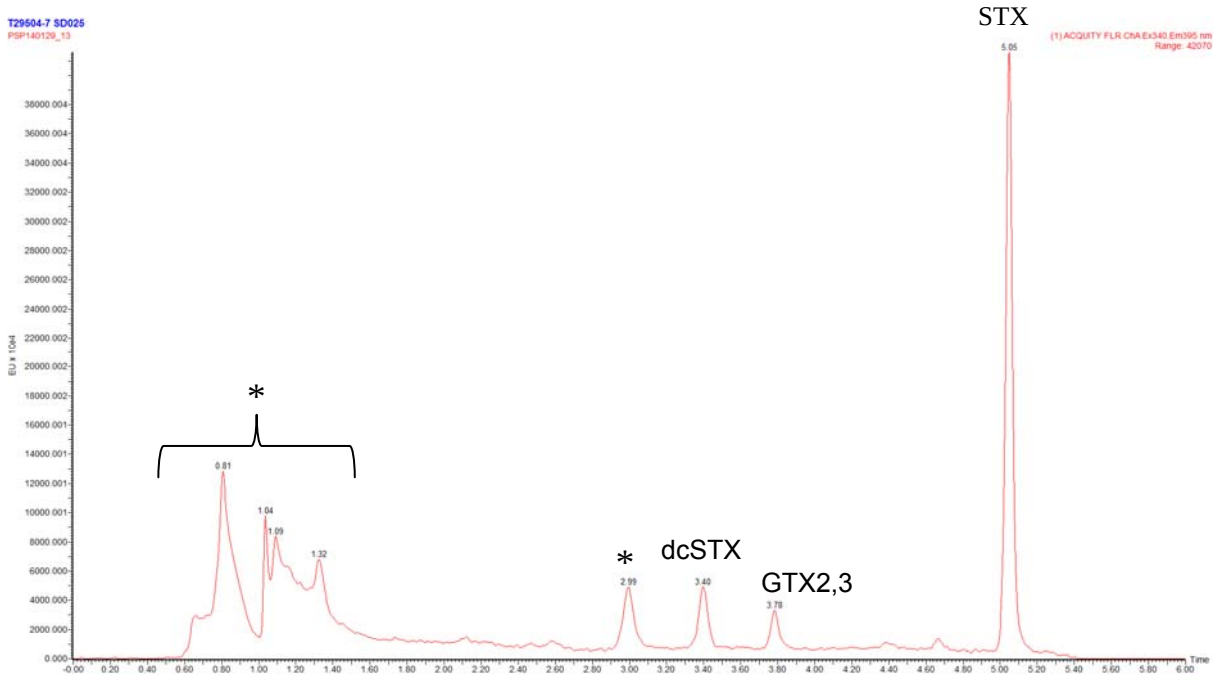

Figure 21. Representative HPLC-FLR screen chromatogram of a contaminated Papamoa (SD025) tuatua sample (*fluorescent matrix components).

During routine screen testing the amount of STX present in a sample (calculated from the late eluting peak) is corrected for a recovery of 59% (RSD 19%). This recovery factor is calculated from the average measured recovery of STX spiked into shellfish matrix (primarily NZ Greenshell™ mussel; 31 of 33 separate analyses).

Experimental

Papamoa Beach tuatua sample T30202-7 (Table 1) clearly gave a confirmation / screen ratio > 1.0. It was retested on separate days by screen (n = 4, mean 0.64 mg STX equivalent/kg) and on three separate days by confirmation (n = 3, mean 1.1 mg STX equivalent). The average confirmation / screen ratio was therefore 1.7 (1.1 / 0.64). The average recovery of STX from seven tuatua samples taken from different locations around New Zealand was 49%, including one from Papamoa Beach (T30729-4) (47%). Repeatability was also tested in natural and fortified samples and was < 8% in all cases. **However, the STX recovery from sample T30202-7 was determined to be only 32% (n = 5, RSD 2.1%) and was excluded because it was significantly lower.**

Discussion

We believe that the reason for the discrepancy in tuatua sample T30202-7 is due to a lower than expected STX recovery in the screen test. It is therefore likely that any other similar high confirmation/screen ratios observed in tuatua from this same site are due to the same problem.

The primary cause of this discrepancy is the unique STX-dominated profile observed in the sample. When STX has poor recovery, not correctly compensated for via the routine 59% recovery factor, this profile results in a confirmation/screen ratio > 1.0. When STX is quantified via confirmation testing, using peroxide oxidation, the result is less susceptible to interference and no recovery factor is applied due to the harsh nature of this reaction. The peroxide reaction destroys *N*-hydroxylated compounds and is therefore not suitable for the screen test. The other tuatua sample from Papamoa Beach to have STX recovery tested (T30729-4) demonstrated an acceptable recovery of 47% and a ratio of 0.85. This result confirms the role of variation in recovery in generation of ratios over 1.0.

During the inter-laboratory testing of this method (Lawrence, 2004) the reproducibility for STX was measured up to 43%, meaning that even the STX results from the confirmation are quite likely to vary significantly. A simple periodate-only screen is significantly cost-effective and allows the assay to be used for routine analysis. This screen test is designed to be highly conservative and report a worst-case result. The conservative nature of the result is due to the way peaks from a mixture of PSP-toxins are assigned. But when all of the toxicity is due to one toxin, especially when this is STX, then in theory the results should be the same and the ratio should be 1.0. Since implementing this investigation additional tuatua samples were subjected to both screen and confirmation testing. In all cases the ratio was < 1.0, expect one sample from Waihi Beach (24/02/14), two samples from Pukehina Beach (16/02/14 and 18/03/14) and one sample from Ohope Beach (10/02/14). The ratios ranged from 1.0–1.2, which is within experimental error.

Conclusion

This investigation has found that the method is operating as expected in both confirmation and screen mode. It was already known that some profiles, particularly those dominated by STX, would potentially produce higher confirmation than screen results due to normal analytical variation. This theoretical possibility has now been shown in practise. **These results confirm the generation of screen results less than the subsequent confirmation result in selected tuatua samples, is due to low STX recovery.** This is most likely related to interference during the periodate oxidation procedure. This does not appear to be a tuatua or site-specific problem. The STX recovery data for NZ Greenshell™ mussels (59%) is appropriate to apply given the general nature of the screen. However, exceptions can occur and these may potentially be due to uncommon sample matrices / contaminants. When these exceptions occur, and when the toxin profile is dominated by STX, a screen result lower than the confirmation result is not an unexpected outcome.

As a result of this investigation, if a sample is found to contain STX at a level ≥ 0.4 mg/kg STX eq (*i.e.* half the regulatory limit), confirmation testing is triggered. This ensures that when a STX-dominated profile is observed the screen test will not underestimate sample toxicity.

7. ACKNOWLEDGEMENTS

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9. APPENDICES

Appendix 1. Reported results from the Cawthron Institute laboratory of paralytic shellfish poisoning (PSP) analysis of Bay of Plenty shellfish, November 2013–June 2014

Sample site	Date sampled	Species	Screen	Confirmation
Tairua	10/02/14	Green-lipped mussel	<0.1	
Waihi Beach	03/11/2013	Tuatua		1.0
	17/11/2013	Tuatua		1.2
	01/12/2013	Tuatua		0.7
	16/12/2013	Tuatua	≤0.47	
	30/12/2013	Tuatua	≤0.68	
	12/01/2014	Tuatua		~0.6
	27/01/2014	Tuatua	≤0.66	
	10/02/2014	Tuatua	≤0.13	
	24/02/2014	Tuatua		0.43
	10/03/2014	Tuatua		0.55
	23/03/2014	Tuatua		0.35
	28/04/2014	Green-shell mussel	<0.1	
	07/05/2014	Green-shell mussel	<0.1	
	18/05/2014	Green-shell mussel	<0.1	
	04/06/2014	Green-shell mussel	<0.1	
Papamoa Beach	03/11/2013	Tuatua		0.96
	17/11/2013	Tuatua		1.4
	01/12/2013	Tuatua		1.3
	16/12/2013	Tuatua		~1.0
	30/12/2013	Tuatua		~0.6
	12/01/2014	Tuatua		~0.8
	27/01/2014	Tuatua		1.4
	10/02/2014	Tuatua		1.4
	24/02/2014	Tuatua		1.1
	10/03/2014	Tuatua		1
	23/03/2014	Tuatua		0.94
	08/04/2014	Tuatua		0.97
	22/04/2014	Tuatua		0.7
	07/05/2014	Tuatua		1.2
	18/05/2014	Tuatua	≤0.56	
	04/06/2014	Tuatua	≤0.46	
Pukehina Beach	12/11/2013	Tuatua		0.69
	25/11/2013	Tuatua		0.89
	09/12/2013	Tuatua		0.49
	22/12/2013	Tuatua		~0.4
	05/01/2014	Tuatua	≤0.26	
	20/01/2014	Tuatua	≤0.76	
	02/02/2014	Tuatua	<0.1	
	16/02/2014	Tuatua		0.32
	02/03/2014	Tuatua		0.15

Sample site	Date sampled	Species	Screen	Confirmation
Pukehina Beach *continued*	18/03/2014	Tuatua		0.21
	30/03/2014	Tuatua		0.1
	14/04/2014	Tuatua	≤0.2	
	28/04/2014	Tuatua	≤0.33	
	13/05/2014	Tuatua	<0.1	
	26/05/2014	Tuatua	≤0.21	
Whakatane Heads	04/11/2013	Greenshell mussel		0.82
	11/11/2013	Greenshellmussel	≤0.4	
	02/12/2013	Greenshellmussel	<0.1	
Ohope Beach	18/11/2013	Tuatua	≤0.17	
	16/12/2013	Tuatua	≤0.11	
	30/12/2013	Tuatua	≤0.24	
	13/01/2014	Tuatua	≤0.14	
	28/01/2014	Tuatua	≤0.32	
	10/02/2014	Tuatua		0.7
	25/02/2014	Tuatua		0.09
	10/03/2014	Tuatua		0.08
	24/03/2014	Tuatua		0.08
	08/04/2014	Tuatua	<0.1	
	22/04/2014	Tuatua	<0.1	
	6/05/2014	Tuatua	<0.1	
	18/05/2014	Tuatua	<0.1	
	02/06/2014	Tuatua	<0.1	
Opape	12/11/2013	Green-lipped mussel	<0.1	
Whangaparaoa	03/11/2013	Greenshell mussel	<0.1	
	19/11/2013	Greenshellmussel	<0.1	
	01/12/2013	Greenshell mussel	<0.1	
	13/01/2014	Greenshell mussel	<0.1	
	28/01/2014	Greenshell mussel	<0.1	
	24/02/2014	Greenshell mussel	<0.1	
	09/03/2014	Greenshell mussel	<0.1	
	23/03/2014	Greenshell mussel	<0.1	
	07/04/2014	Greenshell mussel	<0.1	
	27/04/2014	Greenshell mussel	<0.1	
	11/05/2014	Greenshell mussel	<0.1	

Appendix 2. Screen analysis of tuatua, *P. subtriangulata* samples collected at four localities on Papamoa Beach, Bay of Plenty in the course of hydrocarbon residue monitoring by the University of Waikato, 24 February 2014.

Papamoa Beach sampling sites (24/02/14)	**Pre-column oxidation screen (mg/kg)**
Concord Avenue	≤0.84
Tay Street	≤0.27
Domain Road	≤1.0
Taylors Reserve	≤0.84

Appendix 3. A statistical analysis of the drivers of water column stratification in Opua Bay.

To assess what drives the establishment and breakdown of temperature stratification in Opua Bay, a basic statistical analysis was undertaken. This analysis used a generalised linear model (GLM) from the R software package (RDC, 2014) to test the effect of winds, tides and seasonal forcing factors (*e.g.* air temperature and solar irradiation) on stratification expressed as a potential energy anomaly (PEA). The PEA was calculated for the upper 12 m of the water column using seawater temperature profile data and assuming a constant salinity of 35 parts per thousand. The complete data set recorded at 15-minute intervals by temperature loggers suspended at 3 m depth intervals from the Opua Bay raft between 1 January 1 and 30 April 2012, 2013 and 2014 were used in the statistical analyses. A high positive PEA value is associated with a lower centre of mass for the water column (*i.e.* warmer, lighter water overlying cooler, denser water) and is an indicator of high stability. Low or negative PEA values indicate an unstable situation where the density of the water column is uniform or dense water overlays less dense water that will eventually mix.

Total wind speed (MetService, Cape Campbell; Figure 8) was not well correlated with PEA, so only the northerly (NW) component of the wind was included in subsequent analysis. This is consistent with the approximate north-south orientation of Opua Bay. Raw tidal elevation predictions were not correlated with PEA, consequently only the tidal range (TR) was used in the final model analysis. In order to account for other drivers that varied throughout this period of the year and were not accounted for by wind and tides (*i.e.* solar irradiation, external ocean temperatures and air temperature), a month variable was added as a factor to the model. The distribution of PEA results were skewed, consequently the logarithm of the raw PEA values plus a scalar of five (to avoid negative values) was used in the final model. Initial comparisons undertaken in a simple pair plot are presented in Fig. A3.1. It was apparent from the pair plot correlation results that there were no issues with cross-correlation between potential driver variables (*i.e.* between-driver correlations were low). Consequently all driver variables were included in the final model. The formulation of the final GLM model is described as follows:

$$\text{Log}(PEA+5) = a(\text{Month}) + b.TR + c.NW + d.TR.NW + f$$

Where the abbreviations for the variables are described above and the letters a to d represent the coefficients of the model plus a constant intercept value ‘f’.

The results of the GLM analysis (Table A3.1) showed that approximately 43% of deviance in the PEA was able to be explained by the month, tidal range and northerly winds (*i.e.* $R^2 = 0.428$). The majority of the explained variability of the model was associated with the month factor (*e.g.* solar irradiation and air temperature), which accounted for up to 40.8% of the deviance ($R^2_{\text{month only}} = 0.408$).

Table A3.1. Generalised linear model (GLM) results showing the correlations between time of year, wind and tide and the stratification parameter, potential energy anomaly (PEA), in Opua Bay.

Coefficients	Estimate	Std Error	Probability
February	0.023	0.003	<2e-16***
March	-0.220	0.003	<2e-16***
April	-0.429	0.003	<2e-16***
Tide range	0.141	0.004	<2e-16***
NW wind	0.008	0.001	<2e-16***
Tide range:NW wind	-0.006	0.001	<2e-16***
Intercept	2.048	0.005	<2e-16***

This was not unexpected because stratification is strongest during the warmest months of the year. However it was also clear that the TR and northerly wind forcing (NW) were also important contributors to the stratification state of the region as the size of the coefficients from the model were highly significant ($p < 2e\text{-}16$; Table A3.1). This implies there was greater than 99.9% chance that the estimates were significantly different from zero (*i.e.* uncorrelated). The size of the driver coefficients permit comparison with the effect of month (coefficient differences of about 0.2) in the model. For tidal range differences of about 1 m (*i.e.* 1 × 0.14 = 0.14) and northerly wind strengths greater than about ±20 m/s (*i.e.* 20 × 0.0075 = 0.15), PEA changes were similar to between month related changes.

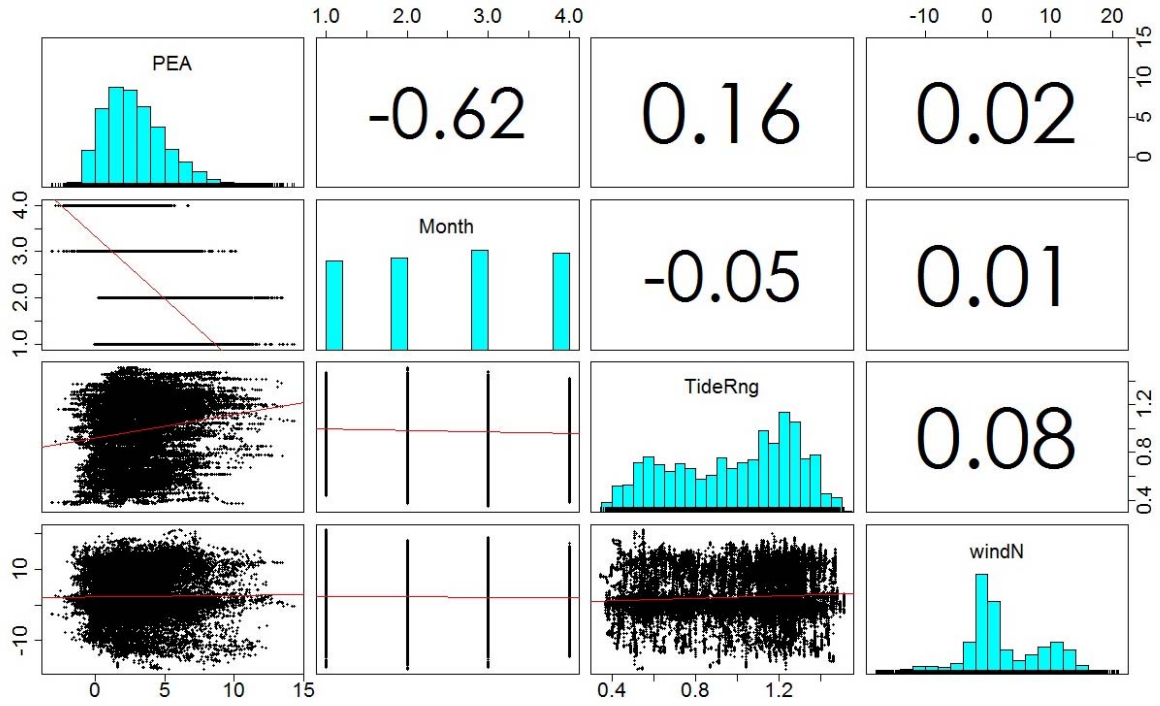

Figure A3.1. Pair plot of potential energy anomaly (PEA) against the potential drivers: month of the year (Month), tidal range (TideRng) and the northerly component of the wind (windN). Numbers shown in the upper right plots show the spearman correlation coefficient values between response (PEA) and driver variables and between driver variables. Histograms of the data used to build the model are shown on the diagonal and the raw data with a simple least squares linear model (red line) are shown in the lower left plots.

Appendix 4. NZ Journal of Marine and Freshwater article: The risk to New Zealand shellfish aquaculture from paralytic shellfish poisoning (PSP) toxins

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REVIEW ARTICLE

The risk to New Zealand shellfish aquaculture from paralytic shellfish poisoning (PSP) toxins

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New Zealand's reputation as a supplier of high quality food products is vital to the national economy; international consumers are acutely aware of food safety issues and markets are increasingly demanding higher standards. Filter feeding bivalves are particularly sensitive to the nature of the environment in which they are grown, and quality assurance is a major preoccupation of the shellfish aquaculture industry. With the exception of a couple of incidents, most notably the *Gymnodinium catenatum* blooms in 2000–2003, paralytic shellfish toxin (PST) contamination has, to date, not had an important effect on the economics and sustainability of the industry. However, the dinoflagellate species responsible for producing these toxins are not uncommon in New Zealand coastal phytoplankton communities, and it is important that awareness of the potential risk is maintained. This review summarises what we know about the causes and incidence of PST contamination from research and monitoring over the last 20 years, since it was first identified in New Zealand. It describes the dynamics of major events and their consequences, and evaluates what is likely to happen in the future as aquaculture expands into new areas with known histories of this problem.

Keywords: paralytic shellfish poisoning; PSP; saxitoxins; dinoflagellates; *Alexandrium* spp.; *Gymnodinium catenatum*; aquaculture

Introduction

Contamination of filter feeding bivalves with paralytic shellfish poisoning (PSP) toxins is a public health hazard and an important quality assurance problem for the New Zealand shellfish aquaculture industry. The toxins involved in PSP are secondary metabolites produced by planktonic dinoflagellates collectively known as paralytic shellfish toxin (PST). These toxins include numerous (> 30) chemical analogues with varying potency, all of which are based on the parent saxitoxin (STX) molecule. They have high affinity for voltage-gated sodium ion channels in nerve cell membranes, inhibiting the generation of nerve impulses and causing the neurological effects, including paralysis, associated with this food poisoning syndrome (Baden et al. 1995). Because STX is amongst the most potent natural toxin known, there is a high level of awareness about the risk of PST internationally, and their levels in seafood (expressed as saxitoxin equivalents) are tightly regulated.

There have been several recent incidents that have highlighted the potential seriousness of this issue and which are especially relevant to the New Zealand shellfish industry. During the week of 10 December 2012, at least 20 people were reported to have become ill from consuming surf clams (tuatua; *Paphies subtriangulata*) collected by recreational gatherers from Bay of Plenty beaches between Mt Maunganui and Papamoa (ESR 2012). A public health notice warning against the harvesting of shellfish in the region because of PST contamination was in force at the time. Seventeen people were admitted to hospital and at least two serious cases required treatment in the intensive care unit for several days. The patients exhibited

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classic symptoms of PSP, shellfish specimens from a batch that caused human poisoning contained high levels of STX, and neoSTX and toxin residues were identified in the urine from an affected individual (T. Harwood, Cawthron Institute, pers. comm. January 2013). This was the most serious documented PSP event that has occurred in New Zealand to date.

Since the summer of 2011 it has been recognised that there is a chronic problem with the occurrence of toxic *Alexandrium catenella* blooms in Queen Charlotte Sound (QCS). This has resulted in widespread contamination of shellfish with PST (MacKenzie et al. 2011, 2012, 2013). *Alexandrium catenella* (MacKenzie et al. 2004) has been a common member of the phytoplankton on the east coast of the North Island for many years, but it was only identified in QCS in 2010. *Alexandrium catenella* may be in the process of colonising the coastal waters of the northern South Island. If it becomes established in the main mussel-growing areas of Port Underwood, Pelorus Sound and Tasman and Golden bays, the shellfish aquaculture industry may have to manage production around annual region-wide closures of two to three months in late summer and autumn.

In August 2012 and September 2013, after an apparent absence from the phytoplankton for at least five years, the PST-producing dinoflagellate *Gymnodinium catenatum* reappeared in samples from the North Island west coast (Manukau and Kaipara harbours, Port Taranaki; Fig. 1) associated with low levels of contamination of shellfish in the region. In 2000–2001, a major bloom of *G. catenatum* led to the widespread contamination of shellfish around much of the North Island. This event was the most extensive, and one of the most economically harmful, toxic algae blooms to be documented in New Zealand to date. There were few accounts of human illness associated with the bloom; however, it led to health authorities issuing warnings to the public to refrain from collecting shellfish from over 1500 km of coastline, for periods of over nine months in some regions. Although the main commercial shellfish growing areas (Marlborough Sounds, Coromandel) were only marginally affected, the bloom had an important impact on the shellfish industry throughout the country. This was due to prohibitions being placed on the movement of juvenile shellfish (mussels and oysters) from affected to non-affected areas, because of the risk of the translocation of *G. catenatum* resting cysts. Unfortunately, much of the pre-mium seed source for the oyster and mussel industries lay within affected areas and, as a result, there was a shortage that influenced production in subsequent years.

In October 2012, the Australian shellfish industry suffered a setback when Japanese health authorities found unacceptable levels of PST in a shipment of blue mussels (*Mytilus galloprovincialis*) originating from the east coast of Tasmania (Campbell et al. 2013). This resulted in a global recall of products from this source and extended closures (up to 100 days) of important bivalve production areas. The shellfish poisoning was the consequence of a widespread, but undetected, bloom of the toxic dinoflagellate *A. tamarense*. This species had been known to be present in this region for some time (de Salas et al. 2001). The event caused an estimated direct revenue loss of around A$6.3 million to the mussel industry, and around A$0.8 million and A$1 million to the scallop and rock lobster fisheries respectively. When an economic multiplier was applied to this loss of revenue, the total economic impact was estimated at approximately A $25.6 million. These losses were attributed to a breakdown in the Tasmanian Biotoxin Management Plan procedures, which resulted in inadequate phytoplankton and shellfish toxin monitoring being carried out in the affected region before and while the shellfish were being harvested. This resulted in failure to detect the *A. tamarense* bloom and the PST in the shellfish, and led to the sale and export of contaminated products.

The Tasmanian event serves as a reminder to the New Zealand industry that complacency about the risks of PSP can have catastrophic effects. New Zealand's reputation as a supplier of high quality and safe seafood provides a competitive advantage (Busby & Seamer 2004) and it is crucial that shellfish products containing unacceptable levels of PST residues do not slip through the surveillance system and appear on local or international markets. To ensure this, participants in the shellfish

Figure 1 Map of the North Island (Te Ika-a-Māui) and northern South Island (Te Waipounamu) showing locations referred to in the text.

industry need to be fully informed about the nature and risks of these phenomena as we currently understand them. The aim of this review is to present a synopsis of what is now known about PST contamination of shellfish in New Zealand, from research and monitoring that has taken place over the two decades since it was first identified. The review will:

- Summarise research on the toxicity and ecology of the dinoflagellate species that are responsible for the production of PST in New Zealand
- Explain changes that have taken place in the toxin monitoring methods.
- Review the history and dynamics of the major PST events that have taken place over the last 20 years.
- Describe the impacts that major blooms have had on the aquaculture industry and the various measures that have been taken to mitigate these effects.

Paralytic shellfish poisoning regulation, testing and monitoring

Agreements between the New Zealand Ministry for Primary Industries (MPI) and major international food safety agencies, such as the US Food and Drug Administration (USFDA) and European

Food Safety Authority (EFSA), permit MPI to manage surveillance and monitoring of export shellfish for algal-biotoxin contamination. Biotoxin regulation and monitoring procedures for commercial shellfish harvesting in New Zealand are specified within the Animal Products (Regulated Control Scheme – Bivalve Molluscan Shellfish) Regulations and Specifications 2006. MPI also administers the monitoring programme that oversees non-commercial shellfish gathering.

Important changes have been made in the last few years to the method used to test for PST in shellfish in New Zealand. Until 2010 all testing was carried out using the standard mouse bioassay (MBA) method (APHA 1970; AOAC 1990). This method quantified PSP toxicity by the time it took mice to die with the characteristic symptoms of PSP, within one hour of injection of acidic shellfish extracts. The prohibited level was > 80 µg saxitoxin equivalents/100 g of shellfish flesh. This method has been used internationally for at least six decades and has a limit of detection of approximately 37 µg STX equivalents/100 g. In 2010, because of technical (false positive and negative results, poor sensitivity) and ethical problems associated with the MBA, including European Union (EU) legislation limiting the use of animals for regulatory food testing, an assessment of alternative analytical methods was made (Holland et al. 2010a). A chemical analysis (the Lawrence HPLC/pre-column oxidation method; Lawrence & Niedzwiadek 2001; Lawrence et al. 2004, 2005) was selected and authorised for routine use by MPI in mid-2010, after a thorough evaluation of its performance (Holland et al. 2010b). An effect-based assay, such as the MBA, provides an approximate measure of the total toxicity of the sample in one step. Chemical analysis requires the quantification of each individual toxin type in the sample (there are >12 common and >20 rarer analogues with varying potencies), calculation of the toxicity (in STX equivalents) contributed by each analogue, and summing of these values to arrive at an estimate of total toxicity. The method is complex, but it is sensitive, and provides accurate estimates of total toxicity (Harwood et al. 2013). All public health and shellfish industry testing is now carried out this way in New Zealand. To simplify the method, and make it more cost effective, a two-tier system has been adopted. This involves a rapid screening method that detects toxins and assigns the analogues to broad categories. Most shellfish samples are negative and this efficiently identifies these, while for those samples that contain toxins it provides an approximate toxicity value. To obtain a definitive estimate of toxicity (if required), a second confirmation analysis needs to be carried out. Estimates of total toxicity critically depend on the toxicity equivalency factors used in the calculations, which are currently being experimentally revised (Munday & Reeve 2013; Munday et al. 2013). Total toxicity is now reported in mg STX equivalents/kg of shellfish flesh. The current internationally accepted regulatory level is 0.8 mg STX equivalents/kg and the Lawrence method has a reporting limit of 0.1 mg STX equivalents/kg (Harwood et al. 2013). In 2009, the European Food Safety Authority (EFSA) carried out a toxicological evaluation of the acceptable level of PST in shellfish (EFSA 2009) based on the Authority's review of reports of human intoxications. It suggested that the acceptable level should be reduced to as low as 0.075 mg STX equivalents/kg, which is more than 10 times less than the current level. However, the EFSA conceded that there were too many uncertainties regarding dietary exposure levels and differences in extraction methods for it to make a definite recommendation. If this lower level was adopted, it would be very harmful to shellfish industry interests. It would be a standard that would be very difficult, if not impossible, to meet in practice because 0.075 mg STX equivalents/kg is below the detection level of currently validated methods (i.e. the MBA and the Lawrence HPLC methods) and would substantially lengthen closure periods. The conclusions of the EFSA have been challenged by Kiermeier et al. (2009) who identified significant weaknesses in several aspects of the EFSA analysis. These included: the paucity and quality of data on exposure of consumers to STX-group toxins; biased consumption data; uncritical evaluation of epidemiological data; lack of peer review; and ignoring the recommendation of the Codex Alimentarius Committee on Fish and Fisheries products (FAO/WHO 2006), which stated

that no change in the current level of 0.8 mg STX equivalents/kg was necessary.

Although there had been some prior research and monitoring aimed at identifying PST in the Marlborough Sounds (MacKenzie 1989), a nationwide shellfish biotoxin monitoring programme did not begin until 1993 (Trusewich et al. 1996). This was as a result of the neurotoxic shellfish poisoning (NSP) crisis on the North Island east coast that year (Chang et al. 1995; MacKenzie et al. 1995a). During the first year of the programme 165–200 sites per week were sampled around the country, at a cost of around NZ$4.3 million/annum. This was scaled back to 120 sites per week for the 1994–1995 year (at a cost of around NZ$3.2 million/annum). This trend has continued as priority areas have been identified and phytoplankton monitoring has played a greater, more cost effective, role in the programme (Todd 1997; Chang 2004; NZFSA 2007; Rhodes et al. 2001, 2013). In 1996, the programme was divided into two: an industry programme run and funded by local Shellfish Quality Assurance Delivery Centres; and a government-funded, non-commercial, public health protection programme. In 2013, shellfish from about 20 sites per week were analysed for PST for the shellfish industry and about eight per week for the public health programme.

Toxic *Alexandrium* species in New Zealand

Of the 25 morpho-species of the dinoflagellate genus *Alexandrium* described by Balech (1995), at least 10 have been identified in New Zealand coastal waters (Cembella et al. 1987; MacKenzie et al. 1996a; Chang et al. 1997; MacKenzie & Berkett 1997; MacKenzie & Todd 2002; MacKenzie et al. 2004). New Zealand isolates of four of these species, *A. catenella, A tamarense, A. ostenfeldii* and *A. minutum*, produce PST in culture (MacKenzie et al. 1996a; MacKenzie & Berkett 1997; MacKenzie & Taylor 2004). However, only *A. catenella*, *A. tamarense* and *A. minutum* have been implicated in actual shellfish poisoning events. These species are well known as the cause of PSP elsewhere in the world (e.g. Moore et al. 2009; Aguilera-Belmonte et al. 2011) and pose the greatest risk to aquaculture. Cultured isolates of *A. ostenfeldii* have included highly toxic and completely non-toxic strains (MacKenzie et al. 1996a). The cysts of this species are common in coastal sediments around New Zealand, but it is only rarely observed within the plankton. This is probably the reason why no PST contamination events have been attributed to it. *Alexandrium catenella* and *A. tamarense* isolates from the North Island east coast are morphologically difficult to discriminate by light microscopy (they differ only by the presence or absence of a ventral pore in the 1st apical plate). Molecular analysis of *A. catenella* and *A. tamarense* isolates from elsewhere in the world (e.g. Lilly et al. 2007; Lilibeth et al. 2012) have shown that these species are a complex which cannot be distinguished on morphological grounds. Likewise, New Zealand isolates of these species have been shown to have identical toxin profiles and LSU rRNA gene sequences (MacKenzie et al. 2004). Records of '*A. catenella*' from this region are, therefore, likely to represent a mix of both morphotypes.

Alexandrium catenella

Cells of *A. catenella* (28–36 µm wide × 22–30 µm long) occur as solitary cells or in pairs and chains of variable lengths of up to 16 cells per chain (Fig. 2A). *Alexandrium catenella* is a prolific producer of benthic resting cysts (hyponozygotes) that are smooth walled and oval shaped. The interior of the cyst is clear with a prominent pigment accumulation body and they are often surrounded by a fragmentary mucilaginous capsule (Fig. 2B). Cells of *A. catenella* have been observed at numerous locations around the North Island coast over the last 20 years, but it is most frequently observed, and generally occurs in highest numbers, on the North Island's east coast from the Bay of Plenty northwards (Fig. 3). All the records of *A. catenella* in the South Island are from the Marlborough Sounds. These records only date from 2010 when the first cells were seen in routine monitoring samples from QCS. However, it is now known, from the distribution of cysts in sediment

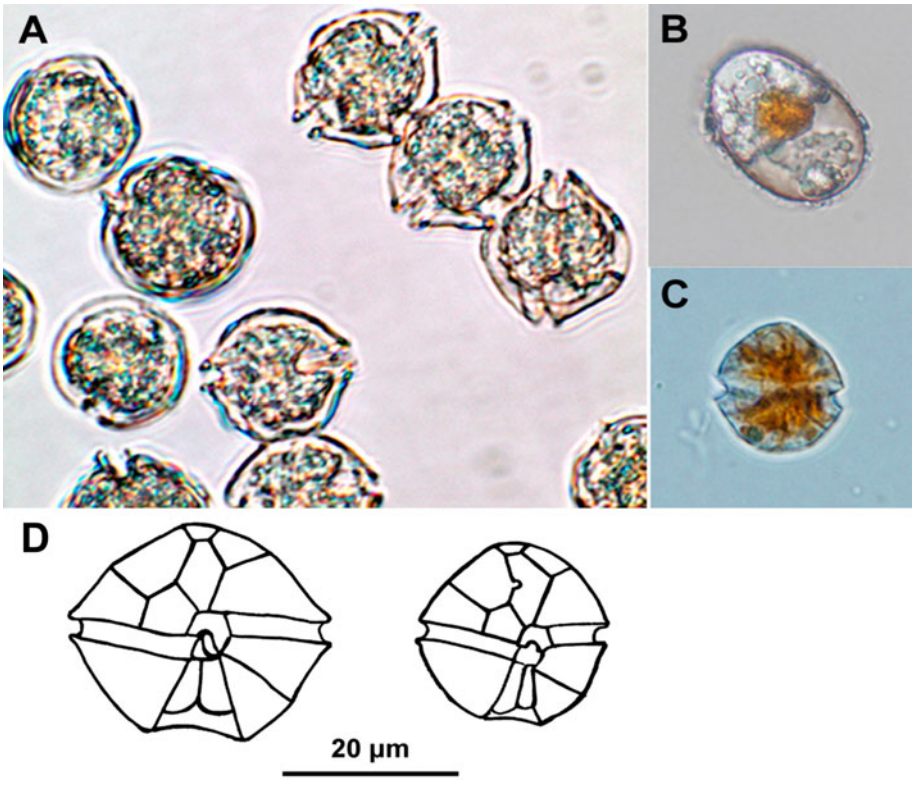

Figure 2 Morphology of *A. catenella* and *A. minutum*. **A**, Motile cells of *A. catenella* in a natural bloom sample; **B**, benthic resting cyst of *A. catenella*; **C**, cultured cell of *A. minutum*; **D**, relative sizes and ventral thecal plate structure of *A. catenella* (left) and *A. minutum* (right).

cores, that this species must have been resident in the region for an extended period before this (see below).

The toxin profiles of wild and cultured cells of *A. catenella* and shellfish that have been exposed to the dinoflagellate have been determined on a number of occasions. MacKenzie et al. (2004) analysed 14 cultured isolates from the Bay of Plenty using the post column oxidation HPLC method of Oshima (1995). The toxin profiles were dominated by almost equal proportions of the low toxicity N-sulfo-carbamoyl analogues (C1,2 and C3,4), with minor amounts of the higher toxicity analogues GTX1,4 and neoSTX. The total mean toxin content of these cells was rather high (150 fmol/cell) but the predominance of the low toxicity C toxins resulted in a low mean specific toxicity (3.4 pg STX equivalents/cell). The toxin profiles of Greenshell™ mussels (*Perna canaliculus*) were very similar to those of the dinoflagellate to which they were exposed (MacKenzie et al. 1997). Analysis of the toxin profiles of *A. catenella* isolates from QCS using the Lawrence pre-column oxidation HPLC method (Harwood et al. 2013; MacKenzie et al. 2013) have shown that these South Island strains also produce c. 50% low toxicity N-sulfocarbamoyl toxins (C1–4 and GTX5–6) but also a high proportion (c. 50%) of the higher potency GTX1–4 toxins (Fig. 4). The toxin profiles of New Zealand strains of *A. catenella* closely resemble those from elsewhere in the world; for example, *A. catenella* isolates from the northeast Pacific (Cembella et al. 1987) and Korea (Kim et al. 2005). Chilean strains of *A. catenella*, reputedly

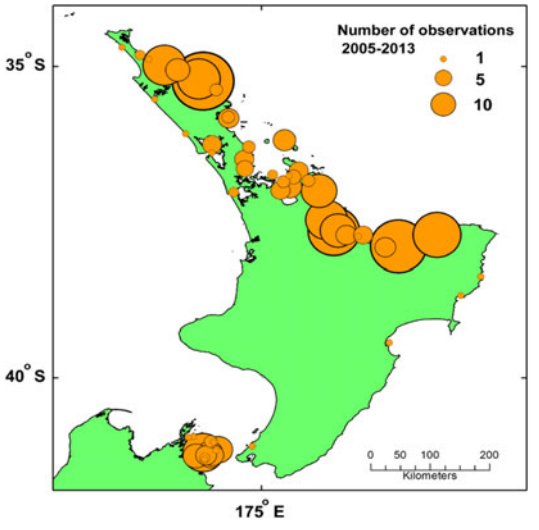

Figure 3 The number of observations of *A. catenella* recorded at weekly phytoplankton monitoring stations, 2005–2013.

responsible for several hundred poisonings and 25 deaths over three decades, are also characterised by the dominance of N-sulfocarbamoyl (C1,2) and gonyautoxin (GTX1,4) analogues (Krock et al. 2007; Varela et al. 2012). However, a genetic and toxicological study (Aguilera-Belmonte et al. 2011) of seven Chilean isolates has shown that some strains had toxin profiles containing significant proportions of high toxicity analogues (neoSTX and STX). Extremely high levels of toxicity (> 127 mg STX equivalents/kg) have been found in Chilean mussels due to contamination by *A. catenella* (Benavides et al. 1995).

Alexandrium minutum

The morphology of Bay of Plenty and Marlborough Sounds isolates of *A. minutum* were described by Chang et al. (1997) and MacKenzie & Berkett (1997) respectively. This species is mainly distinguished from *A. catenella* by its smaller size (Figs. 2C–D). The cells are slightly longer than wide (22–25 µm × 20–21 µm) and generally oval in ventral view, with the epitheca more highly domed than the hypotheca, which is almost hemispherical. There are two other small *Alexandrium* species (*A. angustitabulatum* and *A. camurascutulum*) described from New Zealand waters. These are very similar in general size and shape to *A. minutum* and are unlikely to be discriminated from this species during routine monitoring. *Alexandrium angustitabulatum* was identified (February 1983) from a 'red tide' sample from Whangarei Harbour, Northland, and was shown by Cembella et al. (1987) to produce PST (96% GTX1,4) in culture. *Alexandrium camurascutulum* was described from the Marlborough Sounds (MacKenzie & Todd 2002). It is unknown whether this species produces PST because live specimens have not been

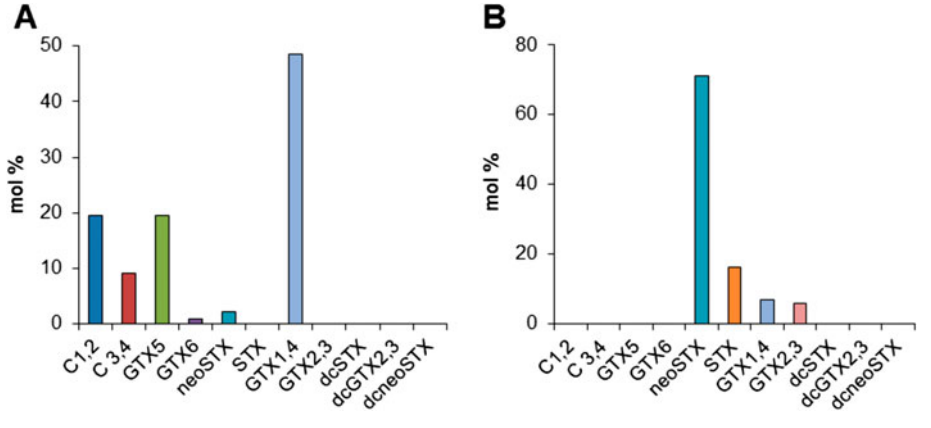

Figure 4 PST analogue profiles of *A. catenella* and *A. minutum*. **A**, Opua Bay *A. catenella* isolate, after Harwood et al. 2013; **B**, Bay of Plenty *A. minutum* isolate, after Chang et al. 1997.

isolated and cultured. Chang et al. (1997) analysed the PST profiles of two cultured isolates of *A. minutum* from the Bay of Plenty (Fig. 4). They found that neoSTX was the principal toxin (> 65 mol%) with STX and GTX1,4 as lesser components. The specific toxicity of these cells was at the high end (6.0–11.5 pg STX equivalents/cell) of the range of toxicity reported for *A. minutum* strains elsewhere in the world. They are somewhat higher than the toxicity of *A. minutum* strains (1.8–2.4 pg STX equivalents/cell) isolated from the Marlborough Sounds (MacKenzie & Berkett 1997). These had toxin profiles dominated by GTX1,4 and GTX2,3 (33 mol% and 23 mol% respectively) but also with significant amounts of neoSTX and STX (18 mol% and 26 mol% respectively) as well. The low toxicity N-sulfocarbamoyl C-toxins (C1–4) or gonyautoxins (GTX5–6) are not produced by any New Zealand *A. minutum* isolates. The greater proportion of the more highly toxic PST analogues in *A. minutum* compared to *A. catenella* may make contamination by this species more dangerous to human consumers. This is the likely reason why the December 2012 shellfish poisoning event in the Bay of Plenty was so severe.

Alexandrium blooms in the Bay of Plenty

A large aquaculture development (Eastern Seafarms Ltd) is underway in the eastern Bay of Plenty. Resource consent has been granted for a 3800 ha mussel farm a few kilometres off the Opotiki coast (Fig. 1). The farm has the potential to produce around 20,000 tonnes of mussels per annum when fully established and it is hoped it will become an important new employment opportunity in the region. This development, which is still in an experimental phase, is in a region that has a chronic problem with PST contamination. As shellfish production comes onstream, special care will need to be taken to ensure that contaminated products do not enter the food chain. The PSP problem in the Bay of Plenty springs from the frequency of blooms of *A. minutum* and *A. catenella*, which result in the periodic widespread contamination of shellfish along the bay's coastline.

The long-term retention of toxin residues in surf clams (tuatua, *Paphies* spp.), which are abundant along the sandy surf beaches of the bay, is a particular problem. These species have always figured disproportionately in national shellfish biotoxin contamination statistics. In the early years of monitoring in New Zealand, large numbers of sites around the entire country were sampled weekly and screened for water soluble and lipophilic algal toxins by MBA. These data provided an excellent nationwide picture of the real magnitude of these phenomena. Between January 1994 and January 1995, 5327 MBAs to detect PSP toxins, were carried out on a variety of shellfish species from an average of 102 sites per week. Of the species analysed, 10% were tuatua. Only c. 3% of the bioassays on all species returned positive results, of which c. 80% were in tuatua, and all but one were the result of low level residues in Bay of Plenty samples. By 1999, after a substantial reduction in the number of monitoring sites, 1660 MBAs were carried out annually. Of these, 8% detected PSP toxins, 85% of which were due to low level residues in Bay of Plenty tuatua.

Over the last 11 years (2003–2013) public health warnings cautioning people against consuming shellfish have been in place for 61 out of 132 months (i.e. 46% of the time) in the Bay of Plenty (Toi Te Ora Public Health Service, Bay of Plenty District Health Board data). Some years (e.g. 2003, 2004, 2005, 2008) only low levels of toxicity have been detected and the beaches have been open for shellfish gathering most of the time. Other years (e.g. 2006, 2010, 2012, 2013) the beaches have been closed the entire year. MacKenzie et al. (1996b) examined changes in the toxicity and PST profiles in populations of *Paphies subtriangulata* from the Bay of Plenty. This was undertaken during the contamination phase caused by a bloom of *A. minutum* in January 1993 and over a six-month period one year later when low level toxin residues persisted in these shellfish. During the peak of toxicity (4.1 mg STX equivalents/kg) the toxin profiles resembled those of *A. minutum*, with a mixture of STX, neoSTX, GTX1,4 and GTX1,3. One year later, when the toxicity had declined to a stable

level of around 0.4 mg STX equivalents/kg, STX was overwhelmingly dominant (94%–100%) and was located almost exclusively in the syphons. The change over time of the toxin profiles and long residence time of toxins in *P. subtriangulata* syphons is similar to the characteristics of other surf clam species elsewhere in the world (Shumway et al. 1994).

Not much is known about the environmental drivers, seasonality and dynamics of *Alexandrium* blooms in the Bay of Plenty, or why there appears to be alternating periods of abundance and shellfish contamination by *A. minutum* and *A. catenella*, but the former may be more common in the western side of the bay and the latter on the eastern side. The only existing data on the spatial distribution of cells in the bay during a bloom were provided by Chang et al. (1996). They first identified *A. minutum* in the western side of the bay in January 1993, as the result of a high cell density (>1.2×10^5 cells/litre) bloom that occurred when surface seawater temperatures and salinities were, respectively, lower and higher than normal for the time of year. Cell numbers of *A. minutum* were highest close to the shore and generally decreased eastwards from Tauranga/Mt Maunganui. They believed the bloom was associated with the nearshore upwelling of nutrient rich waters in the western bay, possibly associated with the El Niño southern oscillation phase persisting at the time.

The first major PSP-toxin contamination event attributable to *A. catenella* occurred in the eastern Bay of Plenty (Whangaparaoa, Ohope Beach) in late March 1996 (MacKenzie et al. 1996c). The toxicity (determined by MBA) in mussels (*Perna canaliculus*) at Whangaparaoa reached a level of around 9 mg STX equivalents/kg. The appearance of toxicity in shellfish progressed from east to west along the shore of the bay and, in late May, tuatua at western sites (Papamoa Beach) showed an increase in toxicity over the normal background. The toxicity in the Whangaparaoa mussels declined rapidly and, by early May, no trace of PSP toxicity could be detected. Typically, the toxicity of the Ohope Beach tuatua declined slowly and was still above the quarantine level in late September.

An *A. catenella* bloom reoccurred the following year and high levels of toxicity (> 10 mg STX equivalents/kg) were found in mussels in the eastern Bay of Plenty (Whangaparaoa, Tokata) in April 1997 (MacKenzie et al. 1997). The contamination from this bloom progressed across the bay westwards as far as Ohope and Pukehina beaches (Fig. 1). Shellfish on beaches further to the northwest (Waihi and Papamoa) remained at normal low background levels. Maximum numbers of *A. catenella* (1.1×10^5 cells/litre) were observed at Te Kaha on 29 April 1997, associated with reports of cloudy, discoloured (brown) water in the area at the time.

Examination of the Bay of Plenty shellfish toxicity data (Fig. 5) shows that significant contamination events are almost an annual occurrence. In some years this is due to blooms of *A. catenella*, in others to *A. minutum*. Blooms can occur in spring, summer or autumn with midwinter being the only time when contamination events are rare. Routine public health monitoring of toxicity and phytoplankton in the Bay of Plenty clearly identified the cause of the December 2012 poisoning incident as due to a bloom of *A. minutum* (Fig. 6). The actual toxicity values in Fig. 6 need to be treated with some caution. These figures represent an HPLC screen test using a method (Assure Qual) designed to alert health authorities to the presence (or absence) of toxins, not their accurate quantification (Jim Sim, MPI, pers. comm. January 2013). Independent analysis (T. Harwood, Cawthron Institute, pers. comm. January 2013) of a tuatua sample from Papamoa Beach at the time of the poisonings using the validated Lawrence method (Holland et al. 2010a,b; Harwood et al. 2013), returned screen and confirmation estimates of 31 and 14 mg STX equivalents/kg respectively.

It is unknown whether the location of the Opotitki mussel farm is susceptible to the influence of these blooms as no testing at the actual farm site has been carried out so far. However, it would be surprising if the farm was not affected to some extent. The Ohope Beach monitoring site, which has a long record of PST-positive samples, is almost directly inshore of the farm. Once the farm is in operation it may only be possible to

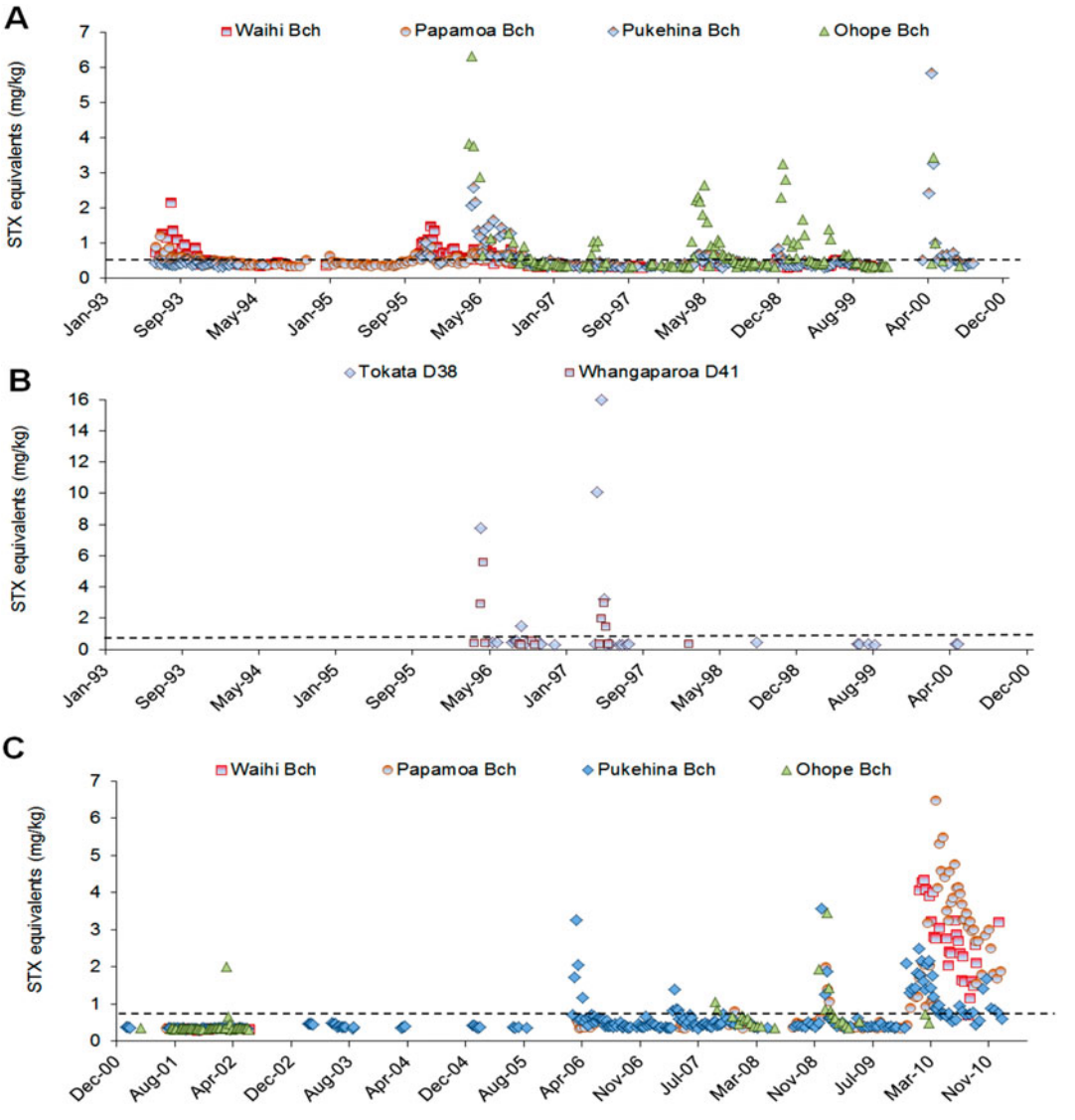

Figure 5 PST levels at monitoring sites in the mid (**A**) and eastern (**B**) regions of the Bay of Plenty 1993–2000 and in the mid region of the bay 2000–2010 (**C**), associated with blooms of *Alexandrium* spp. Tuatua (*Paphies subtriangulata*) were sampled on Waihi, Papamoa, Pukehina and Ohope beaches (**A** and **C**) and Greenshell™ mussels (*Perna canaliculus*) at Tokata and Whangaparaoa (**B**). The toxicity was determined by mouse bioassay. On both graphs the dotted line indicates the regulatory level of 0.8 mg STX equivalents/kg. Negative (i.e. not detected) results are not shown.

harvest during periods between bloom events. To be forewarned of impending blooms, it will be necessary to carefully monitor shellfish and phytoplankton, informed by shoreline testing and knowledge of water movements in the bay. Because of the large size of the farm (3800 ha) a spatially representative sampling protocol will need to be established.

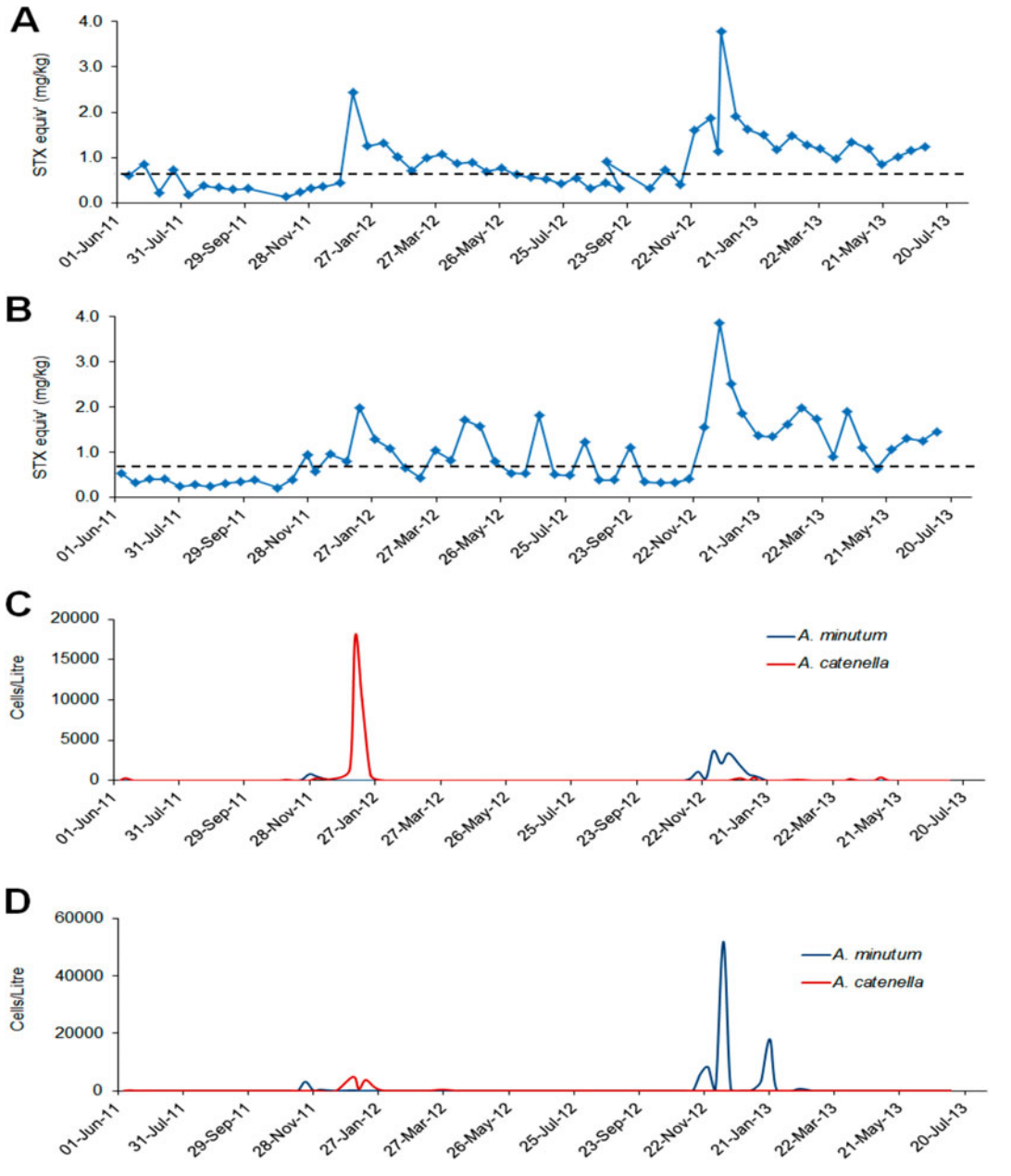

Figure 6 PST levels (**A** and **B**: AssureQuality HPLC screen) in tuatua (*Paphies subtriangulata*) from Bay of Plenty beaches and cell abundance (**C** and **D**) of *A. catenella* and *A. minutum* at Bay of Plenty phytoplankton monitoring sites, June 2011–June 2013. Tuatua sampling sites: Pukehina Beach (**A**) and Papamoa Beach (**B**). Phytoplankton monitoring sites: Tauranga Harbour (**C**) and Bowentown Wharf (**D**).

Alexandrium blooms in the 'Top of the South'

The 'Top of the South' region, encompassing the Marlborough Sounds (Pelorus Sound, QCS, Port Underwood and Croisilles Harbour) and Tasman and Golden bays, is the major shellfish (mainly mussels) growing area in the country. It has an annual production of around 70,000 tonnes/year valued at around NZ$160 million/year in exports. Shellfish aquaculture began in this region in the 1970s, and although there were several minor PST contamination episodes, until 2011 (excluding the *Gymnodinium catenatum*/Kaitaia spat issue; see below) these had little direct effect on the industry.

Alexandrium minutum in the Marlborough Sounds

Occasional sightings are made of *A. minutum* in the Marlborough Sounds phytoplankton, but associated shellfish PST contamination is rare. There have been two minor incidents over the last 20 years, one of which resulted in harvest closures (MacKenzie et al. 1994). During the late winter, spring and summer 1993, *A. minutum* was observed in moderate numbers ($\leq 1.5 \times 10^3$ cells/litre) on several occasions in water samples from Croisilles Harbour. During this period, low level PST-positive mouse assays ($\leq$ 0.4 mg STX equivalents/kg) were detected and there was an association between *A. minutum* at concentrations > 500 cells/litre and this toxicity. In December 1993, *A. minutum* appeared in samples from Anakoha Bay in the outer sounds ($4–7 \times 10^3$ cells/litre), along with positive bioassays for PST in mussels (> 0.5–1.3 mg STX equivalents/kg). A survey of the phytoplankton and bioassay of mussels in the outer sounds showed that low level contamination was confined to Anakoha and Forsyth bays. A limited harvest closure of these areas was imposed for several weeks, but the impact on the industry as a whole was minor.

Alexandrium catenella in Queen Charlotte Sound

Although weekly phytoplankton and shellfish biotoxin monitoring has been taking place at five to six sites in QCS since the early 1990s (Fig. 7), *A. catenella* was not observed in this region until March 2011. At this time, a major bloom developed that resulted in high levels of toxicity in cultured mussels and oysters (Figs. 8–9). This caused a prolonged harvest closure in the area and raised fears that *A. catenella* may spread to the main mussel production areas of the sounds (MacKenzie et al. 2011; Harwood et al. 2013). The first cells were seen in Tory Channel and a low level of toxicity was detected (0.7 mg/STX equivalents/kg) at the Hitaua Bay (Tory Channel) monitoring site in early March 2011. Within one week, cell numbers and toxicity increased substantially at this location. The bloom became more widely dispersed, extending as far as East Bay, where numbers and toxicity peaked (at 1.3×10^5 cells/litre and 17 mg STX equivalents/kg respectively) on 20 April 2011 (Fig. 8B). The suite of PST analogues produced by *A. catenella* isolates from QCS are dominated by low toxicity N-sulfocarbamoyl analogues (principally C1,2) and GTX1,4. Nevertheless, the total PSP-toxin concentrations measured in these shellfish were amongst the highest ever recorded in New Zealand. The dinoflagellate bloom persisted until mid to late April, although the prohibition on commercial shellfish harvesting at East Bay did not end until 20 June 2011. The maximum closure period was 97 days (Table 1). Surveys and monitoring of cell numbers and resting cyst concentrations in the sediments throughout QCS (MacKenzie et al. 2012, 2013) have identified Opua Bay (Tory Channel) as the origin of annually recurrent blooms of *A. catenella*. Very high numbers of planktonic cells ($> 1.0 \times 10^6$ cells/litre) and cysts (up to $1.5–3.5 \times 10^7$ cyst/m^2) have been observed at this location (Fig. 10). Monitoring of the seasonal development of the bloom at this site has enabled predictions of the onset of toxicity at other sites in QCS. A few motile cells of *A. catenella* have been observed in water samples from outer Pelorus Sound and in Port Underwood, but no resting cysts have been found in the sediments of these areas. The dinoflagellate also does not appear to have become established outside QCS as yet.

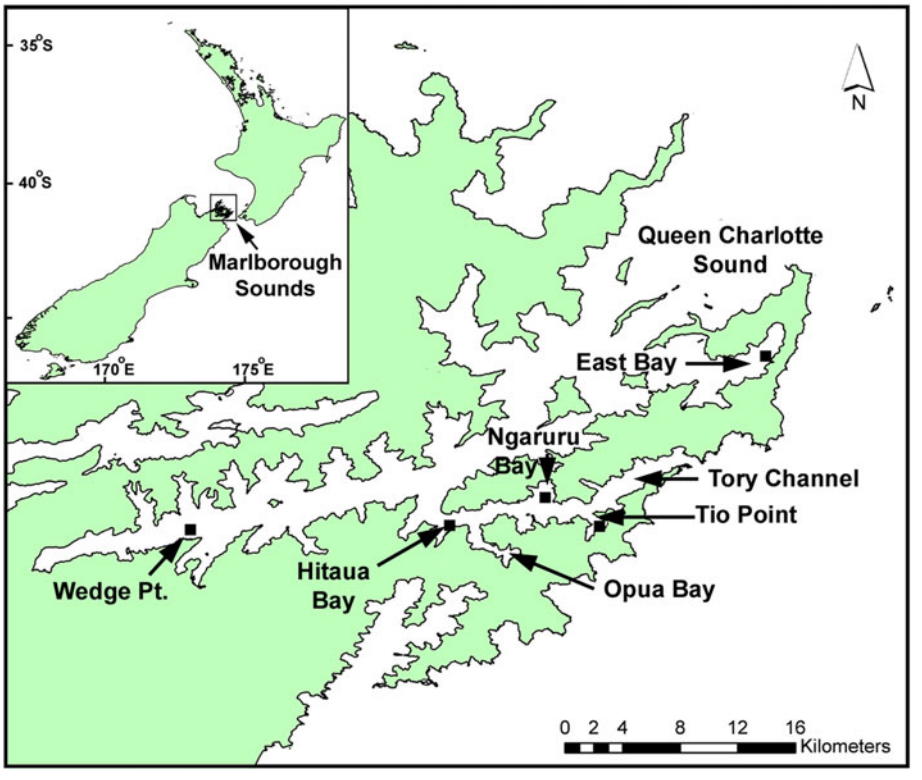

Figure 7 Locations in Queen Charlotte Sound referred to in the text. The small squares mark the location of weekly phytoplankton and shellfish monitoring sites.

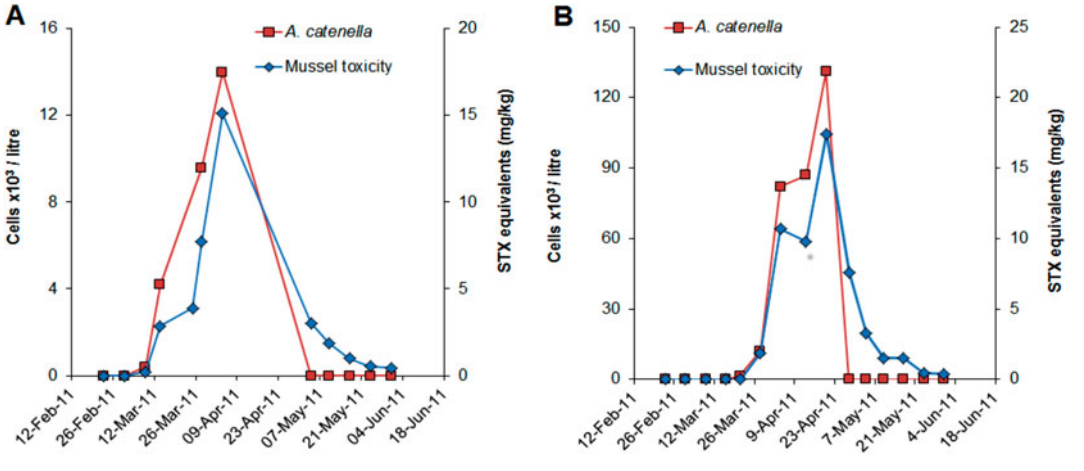

Figure 8 Cell numbers of *A. catenella* (cells × 10^3/litre) in 15 m integrated water column samples and levels of PST (mg/kg STX equivalents/kg) in Greenshell™ mussels (*Perna canaliculus*) at the Hitaua Bay, Tory Channel (**A**) and East Bay (**B**) monitoring sites, February–June 2011. The toxicity levels are adjusted to 'confirmation' estimates by applying a factor of 0.42 to the HPLC screen values.

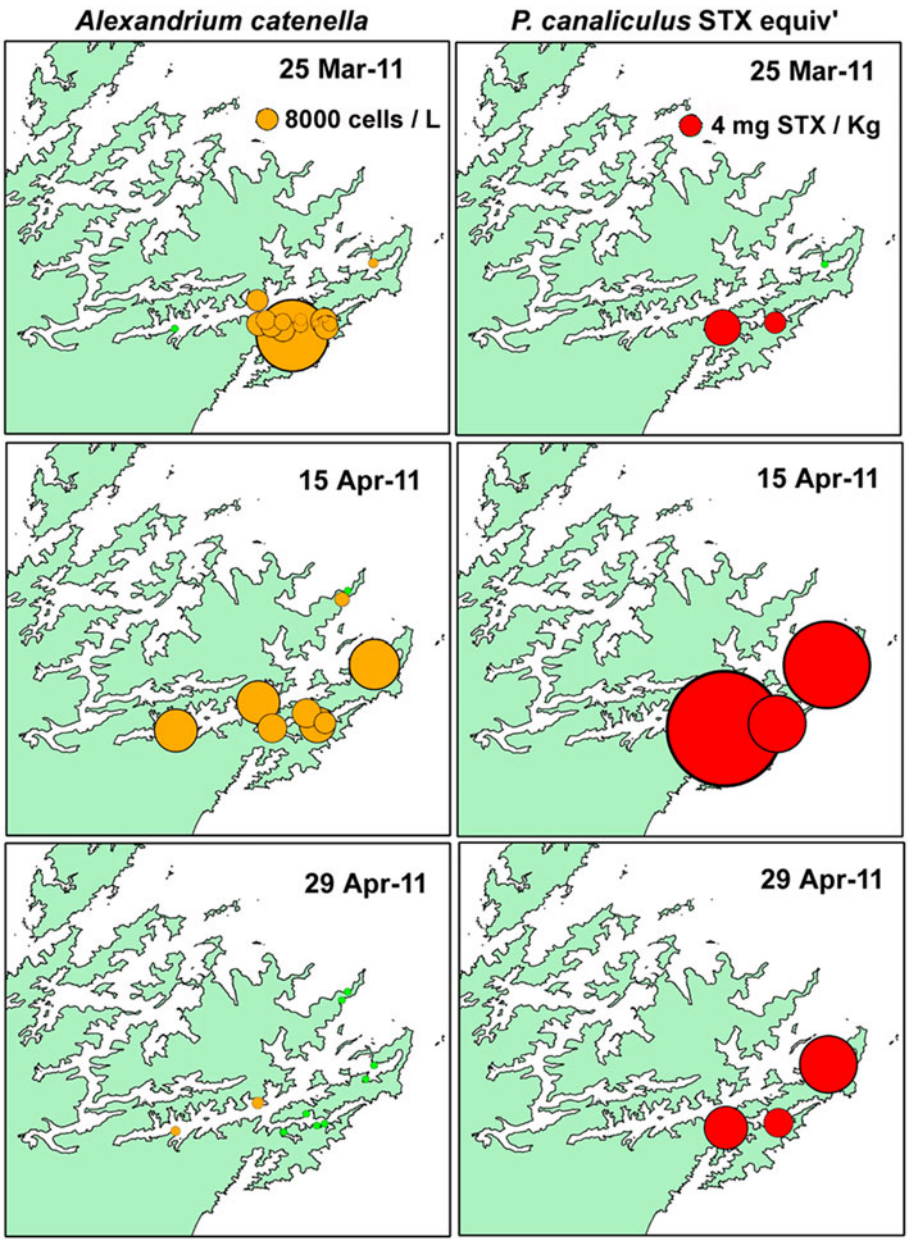

Figure 9 Distribution of *A. catenella* cells and toxicity of Greenshell™ mussels (*Perna canaliculus*) during early (25 March 2011), mid (15 April 2011) and late (29 April 2011) phases of the bloom.

Investigations focused on developing an understanding of the autecology of *A. catenella* in QCS in general, and Opua Bay in particular, have been carried out over the bloom period (January–May) each year since 2011 (MacKenzie et al. 2011, 2012, 2013). The timing of bloom development in Opua Bay, presumably due to cyst germination, is predictable and it is highly likely that they will

Table 1 Shellfish harvesting closure and opening dates in commercial and recreational gathering areas of Queen Charlotte Sound (QCS) due to *A. catenella*.

Location	Close	Open	Duration (days)
Hitaua Bay, Tory Channel	16/03/11	20/06/11	97
Oyster Bay, Tory Channel	23/03/11	17/05/11	56
East Bay	01/04/11	20/06/11	81
Ngaruru Bay, Tory Channel	16/03/11	25/05/11	71
NMDHB closure	25/03/11	21/06/11	89
All QCS	25/03/13	16/05/13	62

NMDHB, Nelson Marlborough District Health Board.

continue to occur every year into the future. Analysis of cyst distribution and isotope dating of strata in sediment cores from Opua Bay suggests that *A. catenella* has been depositing cysts at this location for at least three decades (A.L. MacKenzie, Cawthron Institute, unpubl. data). In Opua Bay, *A. catenella* cells occur in the water column from late spring onwards. However, the bloom does not enter its exponential growth phase until late January to early February, when cell numbers increase rapidly if conditions are favourable. An important factor in bloom development is the establishment and maintenance of a stable, thermally stratified, water column in this sheltered inlet. Opua Bay is exposed to cool fertile waters within Tory Channel. These waters have a high inorganic nutrient content (especially of nitrate) all year, which originates from the deep offshore waters of Cook Strait (Marlborough District Council water quality data). The magnitude of the *A. catenella* blooms in Opua Bay are affected by year-to-year changes in physical and biological conditions within the water column. During the 2011–2012 summer, bloom development was suppressed by strong southeasterly winds in late February and early March, which lowered water temperatures and disrupted water column stratification. From late March to early April, *A. catenella* increased in abundance again as stratification became re-established. It did not become dominant again because of competition from a co-occurring non-toxic dinoflagellate (*Akashiwo sanguinea*) and the lateness of the season. The bloom did not become established to any great extent beyond Opua Bay and no shellfish closures were necessary that year. In contrast, Marlborough experienced an exceptionally fine and sunny summer in 2012–2013. This created the ideal conditions (high light, stable stratified water column) that resulted in the proliferation of *A. catenella* in Opua Bay. An intense bloom (with cell concentrations exceeding 1.0×10^6 cells/litre) developed in mid-March throughout the bay, causing extensive areas of visible 'red tide'. The bloom extended to other areas of QCS, and the timing of shellfish contamination at these locations was similar to that in 2011, but the magnitude and duration was less. In Tory Channel (Hitaua Bay) and East Bay, harvesting was suspended from 25 March to 16 May (71 days). The toxicity at sites affected by Tory Channel water flows were probably due to the export of cells from Opua Bay. At East Bay, however, it is likely that the bloom developed independently from cyst beds that are known to exist there (Fig. 10).

Although *A. catenella* has clearly been resident and blooming annually in isolated embayments of QCS for at least several decades, it is unknown why, within the last few years, it has apparently extended its range. Weekly phytoplankton monitoring has been in progress in QCS since the early 1990s and it is unlikely that it would have escaped attention if it had been present in significant numbers within the wider Sound over that period. The situation in QCS is similar to that in Puget Sound (Washington State, USA). In this area, the link between *A. catenella* and an associated PST contamination has been known for centuries (Trainer et al. 2003) and routine monitoring has been in place since the 1940s. There are frequent closures of commercial and recreational shellfish harvesting in Puget Sound. In the 1980s, PST spread into previously unaffected areas in southern Puget Sound, which contains some of the most productive recreational and commercial shellfish regions on the US west coast. Large-scale oceanographic changes (Pacific Decadal Oscillation) and increased eutrophication

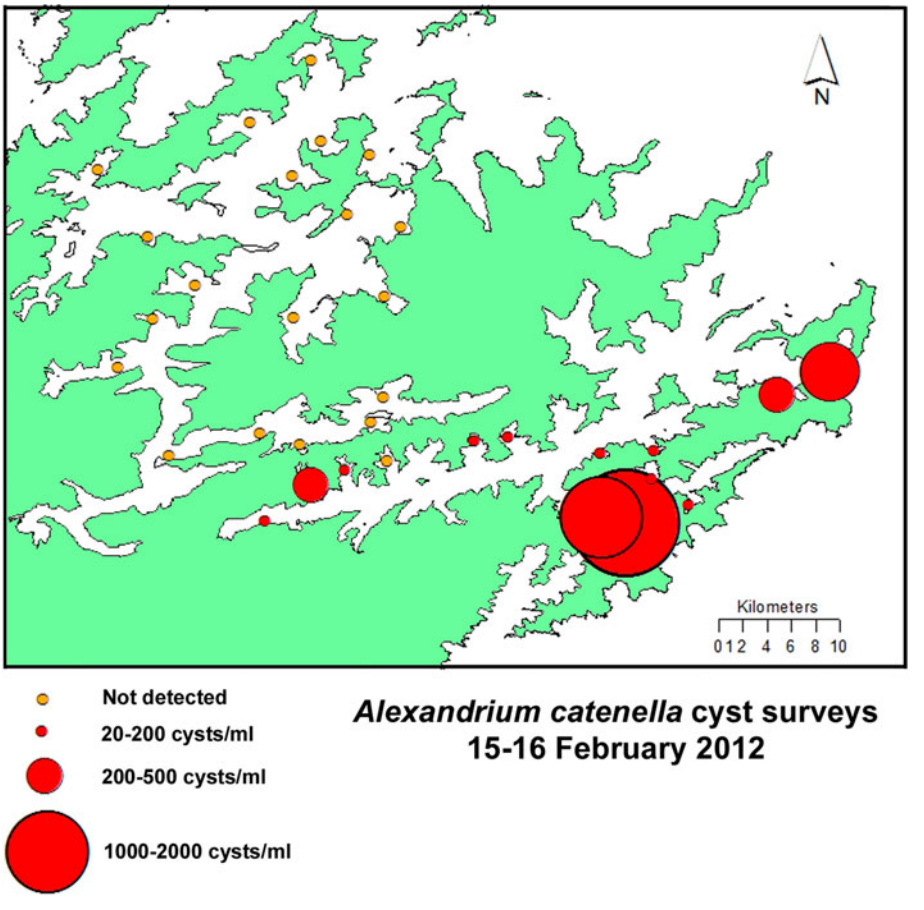

Figure 10 Distribution of *A. catenella* cysts in the surface sediments (0–1 cm) of Pelorus and Queen Charlotte sounds.

of nearshore areas were suggested as possible reasons for the increased problem with PSP in this region. Both QCS and Puget Sound are complex fjords with numerous enclosed embayments off the main waterways, where high resting cyst numbers are found in the sediments and where *A. catenella* thrives under summer stratified conditions. Sequim Bay and Quartermaster Harbour in Puget Sound are two embayments that have high cyst densities in the sediments (Cox et al. 2008; Tobin & Horner 2011; Feifel et al. 2012) and are the origin of annually recurrent blooms. Sequim Bay is flooded by freshly upwelled, high nitrate water from the Straits of Juan de Fuca, similar to the way that Opua Bay receives high nitrate water from Tory Channel.

Alexandrium catenella/tamarense blooms have been associated with fish deaths on salmon farms in Canada (Cembella et al. 2002), the Faroe Islands (Mortensen 1985) and Chile (Fuentes et al. 2008). In the Aysén region of Chile in 2002, a bloom of *A. catenella* caused over 50 human cases of illness, three fatalities and losses of > 1800 tonnes of farmed salmon (Fuentes et al. 2008). In 2006, a reoccurrence of the bloom again caused salmon losses of around 1800 tonnes valued at US$9.2 million. During bloom episodes, *A. catenella* has been observed within salmon farms in QCS, but

cell numbers have generally been low and no adverse effects on the health of the fish have been reported.

Gymnodinium catenatum in New Zealand

Gymnodinium catenatum is the only non-thecate, gymnodinoid dinoflagellate known to produce PST. It occurs in temperate and tropical regions worldwide (Hallegraeff et al. 2012) and has caused problems for shellfish aquaculture in a number of countries (e.g. Spain, Portugal, Tasmania, Japan, Mexico), including New Zealand. For example, in Spain, *G. catenatum* was first detected as the cause of a PSP epidemic in several European countries after people consumed commercially exported mussels from the northwestern Spanish Rias (Luthy 1979). This led to the initiation of shellfish and toxic phytoplankton monitoring in Spain.

In May–June 2000, *G. catenatum* was first observed in seawater samples from the North Island's west coast and PST were detected in shellfish from this region. This signalled the beginning of a major bloom which led to the widespread contamination of shellfish around much of the North Island (MacKenzie & Beauchamp 2011). This was the most extensive, and one of the most harmful, toxic algae blooms to be documented in New Zealand to date. *Gymnodinium catenatum* persisted in some areas (e.g. Kaipara Harbour, Hawke Bay) for several years after the major blooms in 2000–2003, but its incidence gradually dwindled until it appeared to vanish from the plankton in 2007. In August 2012 and September 2013 it again reappeared in samples from the North Island west coast (Manukau and Kaipara Harbours, Port Taranaki), associated with low levels of PST in shellfish in the region. It is likely that, in the future, a repeat of the extensive bloom of early 2000 will take place and the shellfish industry will again confront biosecurity issues regarding the transport of mature and juvenile shellfish around the country. It is important that the aquaculture industry is informed about what is known about the dynamics of the blooms that took place then, their consequences and the management measures that were taken to mitigate these effects.

Gymnodinium catenatum morphology

The morphology of motile vegetative cells and resting cysts of New Zealand strains of *G. catenatum* are identical to descriptions of this species (e.g. Blackburn et al. 1989) from elsewhere in the world (Fig. 11). In nature and in culture it exists as solitary cells or, more usually, in chains of variable length of eight to 16 cells and up to 64 cells per chain (Fig. 11A). The vegetative cells can become swollen and distorted after preservation with Lugol's iodine, but despite this are usually identifiable by skilled observers. In some cases, preserved cells can be confused with cells of other non-toxic chain-forming gymnodinoid dinoflagellates such as *Gymnodinium impudicum* and, when low numbers of solitary cells are present in preserved samples, definitive identification can be difficult on morphological grounds alone. The spherical resting cyst (hypnozygote) of *G. catenatum* is 45–50 µm in diameter (Figs. 11B–D) and has a prominent red pigment accumulation granule within the globular cytoplasm. The surface of the cysts has a distinctive honeycomb-like reticulate pattern that is visible with phase contrast microscopy (Fig. 11D).

Development and progression of the 2000–2001 bloom

Contamination of mussels with low levels of PST (0.35 mg STX equivalents/kg) were first detected in samples collected on 15 May 2000 from the Manukau Harbour (northwest coast of the North Island) during a monitoring programme undertaken by the New Zealand Ministry of Health (Fig. 1). Toxicity was also detected in shellfish samples from Maunganui Bluff and in Raglan Harbour on 20 June, before the first seawater samples were collected from the Manukau on 20 June, within which *G. catenatum* cells were observed (MacKenzie & Adamson 2000). From then, the spatial extent and frequency of sampling was increased and it became apparent that the bloom was intensifying and expanding, both in terms of cell numbers and shellfish toxicity levels (Figs. 12–13). The results of the shellfish toxin assays show the spread and the

Figure 11 Morphology of *G. catenatum*. **A**, Chains of motile cells; **B**, resting cyst (hypnozygote) showing the prominent red accumulation body; **C**, *G. catenatum* cysts in mussel faeces; **D**, ruptured cyst under phase contrast microscopy showing the distinctive 'honey-comb' pattern on the cell wall.

waxing and waning of toxin levels around the North Island coast (Fig. 14). The highest level of PSP toxicity recorded was 40.3 mg STX equivalents/kg in Greenshell™ mussels (*Perna canaliculus*) from Waimamaku in Northland (Fig. 1). Of all the positive bioassays attributable to *G. catenatum* contamination (a total of 537 up to 9 February 2001), 17.5% and 7.3% were at levels > 5.0 and > 10.0 mg STX equivalents/kg respectively.

In general, the appearance of *G. catenatum* in water samples was concurrent with, and often a predictor of, the appearance of PST in shellfish. However, a close correlation between cell numbers and shellfish toxicity cannot be expected on exposed coasts where water sampling is difficult. Along much of the affected coastline, most of the water samples were collected within the surf zone and are therefore not truly representative of cell abundance in the adjacent water column. Nevertheless, cell numbers > 1.0×10^3 cells/litre were common, while at any given site the cell numbers in surf zone samples could vary widely (e.g. from undetectable to 2.7×10^5 cells/litre) in subsequent weeks at one location.

The northern extent of shellfish contamination was the north end of Ninety Mile Beach. Evidence from sampling stations at Spirits Bay and Parengarenga (Fig. 1) showed that the bloom did not spread around Cape Reinga to affect the northeastern coast. The bloom spread steadily southwards along

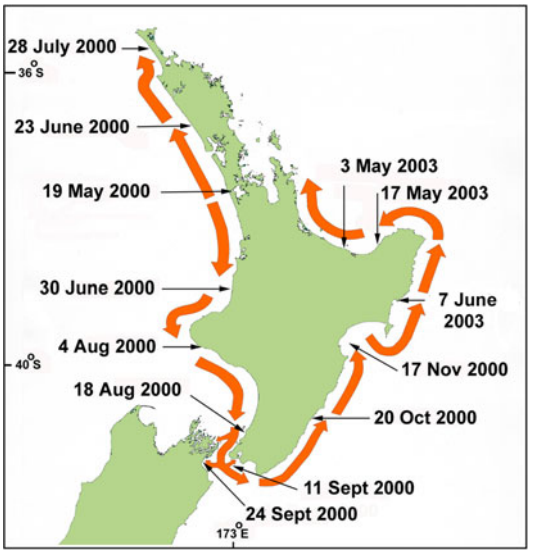

Figure 12 The progression of *G. catenatum* range expansion (dates of first sightings) around the New Zealand coast line, May 2000–June 2003.

the west coast and, by mid-June, it had reached a point just north of Cape Egmont. One week later, shellfish on beaches south of Cape Egmont were showing contamination and, by late August, it had reached Porirua near the southern tip of the North Island. By late September, contamination appeared in Wellington Harbour. From Wellington its northward passage up the east coast was rapid, probably assisted by the north flowing Wairarapa coastal current, until it appeared in Hawke Bay in early November. By mid-November high levels of PST (> 10.0 mg STX equivalent/kg) were detected in this area (Fig. 15). From mid-September to early December 2000, *G. catenatum* cells were often observed in Cook Strait and in waters around the offshore margins of the Marlborough Sounds. No cells were seen within the major mussel farming area of Pelorus Sound. Because of the discovery of low numbers (500 cells/litre) of *G. catenatum* cells in the water and resting cysts in oyster cages and on mussel ropes in Port Underwood and Tory Channel in late September 2000, the industry placed a voluntary ban on the movement of spat and mature product from these areas. No PST was detected in shellfish and, fortunately, *G. catenatum* populations failed to become established in these areas. After surveys of water, sediments and growing structures in Port Underwood did not detect any sign of *G. catenatum* motile cells or live cysts, the ban was lifted in late January 2001.

Gymnodinium catenatum blooms in 2003

Gymnodinium catenatum cells reappeared again in a seawater sample from Kawhia Harbour (west coast of the North Island) at the end of May 2003, and PST appeared in mussels at Mohakatino (North Taranaki) in late June. There were two bloom episodes in the north Taranaki region in 2003, the first peaking in early August and the second in mid-November (Fig. 16). It is possible this was due to inshore/offshore excursions of bloom-affected waters. Data on the spread of the bloom from this region is less comprehensive than that collected in 2000 because there were fewer sites, sampled less frequently. However, a pattern of a gradual intensification of the bloom northwards into the Manukau Harbour and along the northwest coast of Northland was apparent (Fig. 16). High levels of toxicity (22.4 mg STX equivalents/kg) occurred in shellfish from Hui Bank, Manukau Harbour on 17 October 2003. By mid-November 2003, PST (10 mg STX equivalents/kg) were found in tuatua from Ninety Mile Beach. Low numbers of *G. catenatum* cells were observed in Wellington Harbour in mid-October and low levels of toxin were detected in shellfish from lower North Island sites (Foxton Beach, Paraparaumu, Porirua) in October, although the bloom did not reach the intensity of the one in 2000 in this region. On 3 May 2003, *G. catenatum* cells (6.4×10^3 cells/litre) were observed in seawater samples from Ohope Beach, Bay of Plenty. *Gymnodinium catenatum* persisted in samples from this location until 31 May 2003 and, on one occasion (17 May 2003), was also seen in samples from Te Kaha in the eastern bay. This was the first record of a bloom (albeit a minor one) of *G. catenatum* in the Bay of Plenty, although some cells had previously been discovered in a water sample

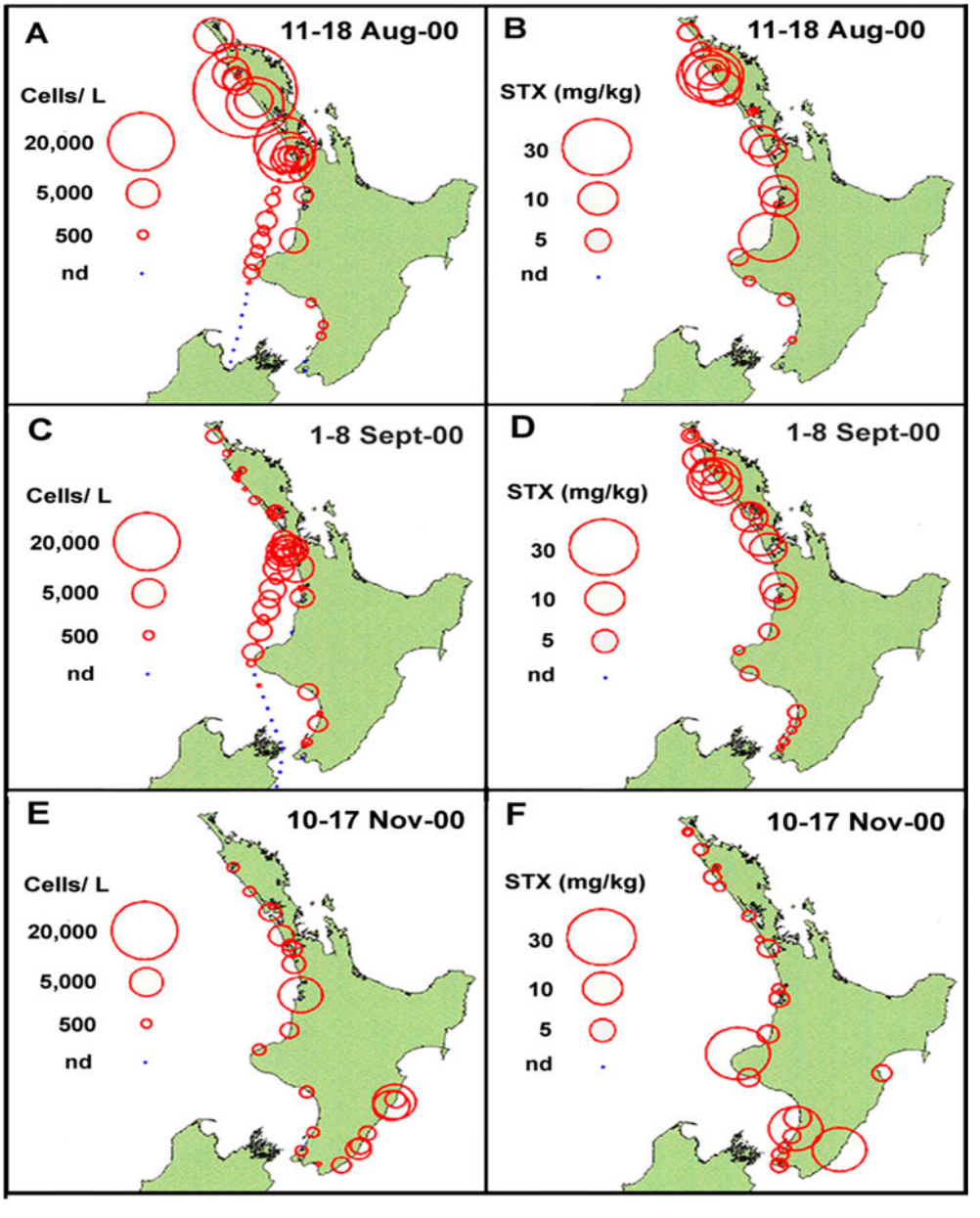

Figure 13 The distribution of *G. catenatum* cell numbers (**A**, **C**, **E**) and PST contamination (**B**, **D**, **F**) in shellfish around the North Island coast, August–November 2000. **A** and **C**, show the cruise tracks and distribution of cells in samples collected offshore from the MV *Spirit of Resolution*.

taken from the Port of Tauranga on 3 December 2000 (Taylor & MacKenzie 2001). *Gymnodinium catenatum* was present at a maximum cell density of 2.5×10^4 cells/litre in samples from Pania Reef (Hawke Bay) in May 2003, where it persisted until late June. Low cell numbers were also seen in samples from other east coast sites (Tolaga Bay in the north to Waimarama in the south) at that time.

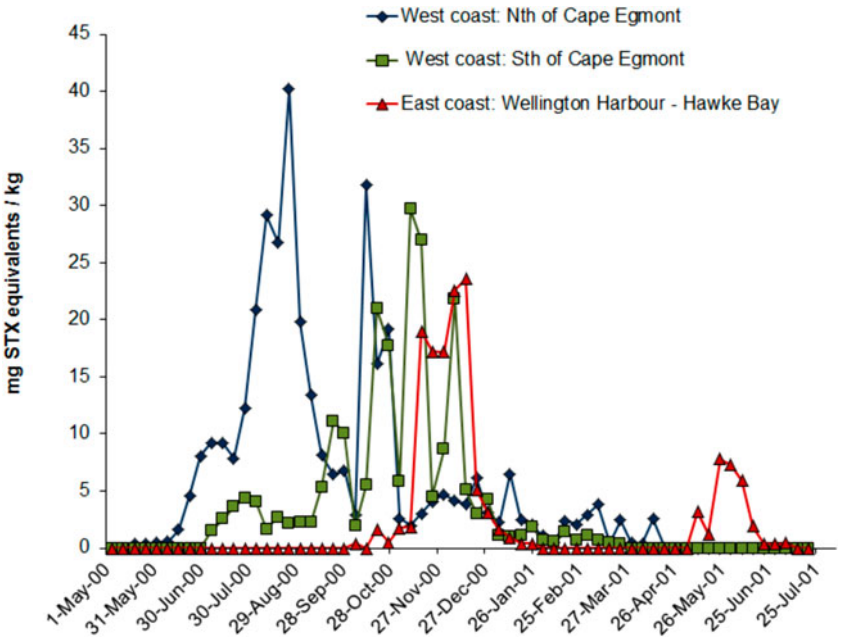

Figure 14 Maximum PST scores determined by mouse bioassay (mg STX equivalents/kg) in shellfish around the North Island coast, May 2000–July 2001. The coast has been divided into three sections; the west coast north of Cape Egmont (13 sampling sites), the west coast south of Cape Egmont (seven sampling sites) and the east coast including Wellington Harbour (five sampling sites). Within these regions, the weekly maximum levels of PST have been plotted.

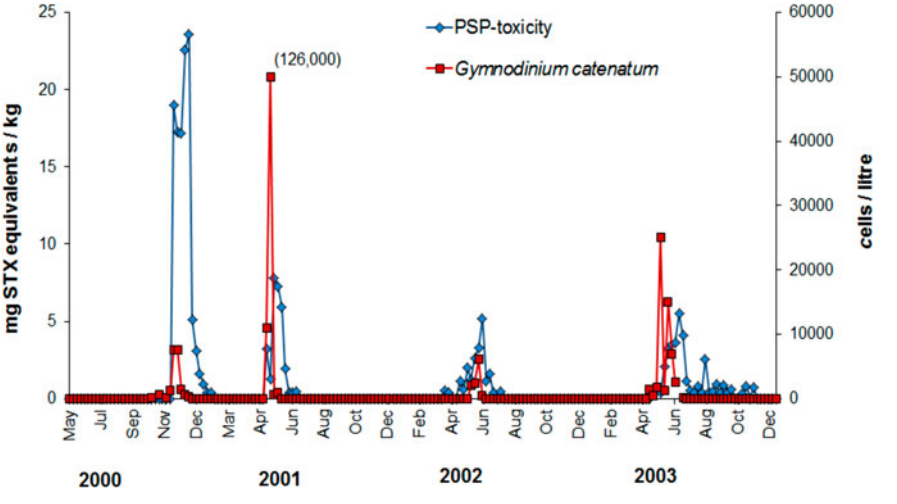

Figure 15 Maximum *G. catenatum* cell numbers and PST levels determined by mouse bioassay (mg STX equivalents/kg) in mussels from Hawke Bay and Wairarapa coastal monitoring sites (Napier, Portland, Blackhead, Whareama), 2000–2003.

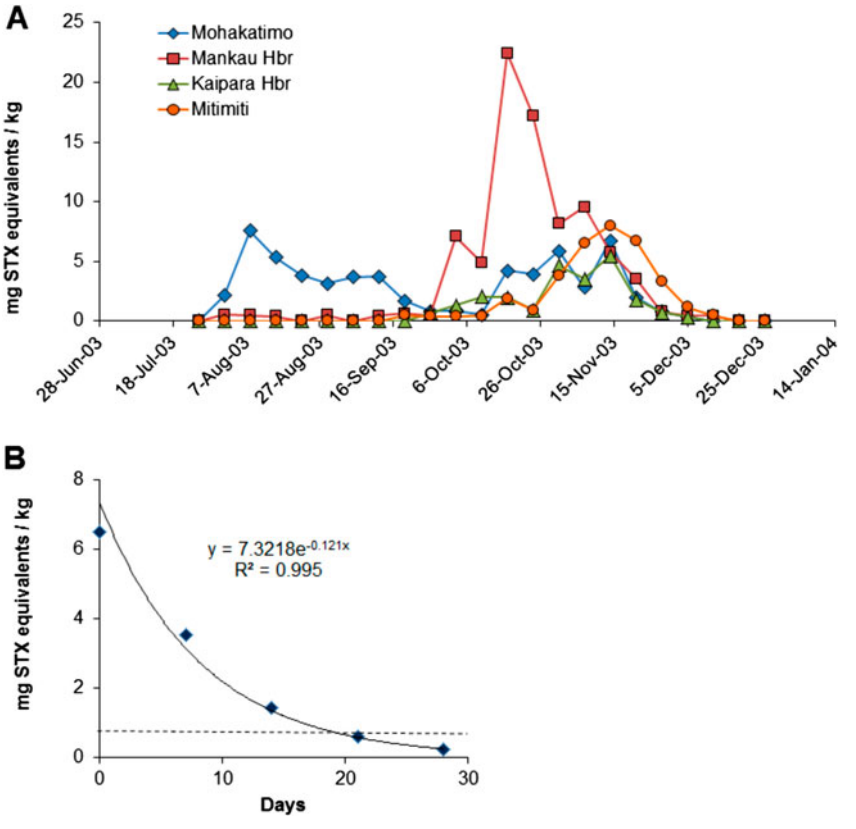

Figure 16 **A**, PST contamination of Greenshell™ mussels (mg STX equivalents/kg) caused by *G. catenatum* from selected North Island west coast monitoring sites, July–December 2003; **B**, depuration curve derived from data from all sites from 15 November 2003 onwards, the half-life of STX in the mussels derived from this curve is 5.7 days. The dotted line represents the maximum accepted toxicity level of 0.8 mg STX equivalents/kg.

Gymnodinium catenatum in Hawke Bay

A resource consent has been granted to Napier Mussels Ltd for the development of a large (2548 ha) offshore marine farm in Hawke Bay. The development is still in an experimental phase, but it is hoped it will be an important economic opportunity for local iwi (Ngāti Kahungunu) who are major shareholders. Because of the history of *G. catenatum* blooms in this region (Fig. 15), it is important that this risk is recognised. The appearance of *G. catenatum* in this area in 2000, and its reappearance for several years thereafter, suggests that for a few years the dinoflagellate established a reservoir of cysts that led to the subsequent blooms. However, the dinoflagellate apparently did not become permanently entrenched in this region, as the last cells were observed in monitoring samples from Hawke Bay in 2007.

Harvest closures due to *Gymnodinium catenatum*

There have been relatively few closures of commercial mussel-growing areas due to *G. catenatum*; however, extensive areas of the North Island coast were closed to recreational harvesting on several occasions during 2000–2004. After a PST contamination event the New Zealand Marine Biotoxin Management Plan specifies that there has to be two consecutive weeks of PST levels below the

regulatory action level of 0.8 mg STX equivalents/kg before an area can be reopened again for harvesting. To obtain an indication of how long Greenshell™ mussel cultivation areas may be closed if they become contaminated with *G. catenatum* toxins, the duration of harvesting closures for wild Greenshell™ mussels in various North Island areas are tabulated (Table 2). An annual closure period of 70 days (about the average for Greenshell™ mussels) in a major growing area such as Pelorus Sound would have a significant economic impact. The toxins produced by *G. catenatum* are water soluble and are rapidly released from mussel tissues with a half-life of about six days (Fig. 16). This is much shorter than the lipid soluble marine biotoxins (e.g. yessotoxin) that are slowly eliminated from mussels with half-lives of up to 50 days (MacKenzie et al. 2002). From the clearance curve in Fig. 16B the approximate depuration time to reach the 0.8 mg STX equivalents/kg level can be calculated according to the equation:

$$\text{Time to clearence (days)} = \log_e(0.8/\text{initial concentration in mg STX equiv}'/\text{kg})/-0.121$$

For example, maximum contamination levels of 10, 5 and 2 mg STX equivalents/kg would take, respectively, about 21, 15 and 8 days to reach the clearance level of 0.8 mg STX equivalents/kg.

Sampling from commercial shipping during *Gymnodinium catenatum* blooms

In September 2000, as the bloom was rapidly spreading southwards along the Taranaki coast, and was approaching the main mussel growing areas within the Marlborough Sounds, a water sampling programme was initiated utilising two of the Cook Strait ferries (Strait Shipping Ltd). Routine sampling of surface waters on the Wellington to Nelson and Wellington to Picton routes of the MV *Straitsman* and MV *Suilven* respectively, commenced in mid-September 2000 and were carried out en route every two to three days until March 2001. Due to the reappearance of *G. catenatum* in Hawke Bay in May 2002, sampling recommenced at weekly intervals on the MV *Straitsman* between Wellington and Nelson on 7 May 2002 and continued until 13 August 2002. Samples were collected using a small bucket on a rope at approximately 10–12 km intervals along each ship's route while the vessels were underway (Fig. 17). This sampling proved useful in rapidly providing data on the distribution of *G. catenatum* cells in offshore waters and in the vicinity of the Marlborough Sounds (Fig. 17; Tables 3–4).

In the samples collected from the MV *Straitsman* on its voyages between Wellington and Nelson, the highest cell numbers were usually found in Wellington Harbour and from mid-Cook Strait eastwards. High numbers were also frequently

Table 2 Examples of the duration of Greenshell™ mussels harvest closures[a] as a result of contamination by PSP-toxins from *G. catenatum*.

Location	Date of closure	Duration
Kaipara Harbour	26/09/03–05/12/03	70 days
Manakau Harbour	03/10/03–12/12/03	70 days
Raglan Harbour	26/09/03–12/12/03	77 days
Kawhia Harbour	10/10/03–28/11/03	49 days
Mohakatino	25/07/03–12/12/03	140 days
Hawke Bay (Pania Reef)	01/11/01–26/01/01	87 days
Hawke Bay (Pania Reef)	11/05/01–29/01/01	49 days
Hawke Bay (Pania Reef)	31/05/02–26/07/02	56 days
Hawke Bay (Pania Reef)	30/05/03–01/08/03	60 days

[a]These are the times from when mussels first exceeded the regulatory level of 0.8 mg STX equivalents/kg until the shellfish had passed two clearance tests at <0.8 mg STX equivalents/kg.

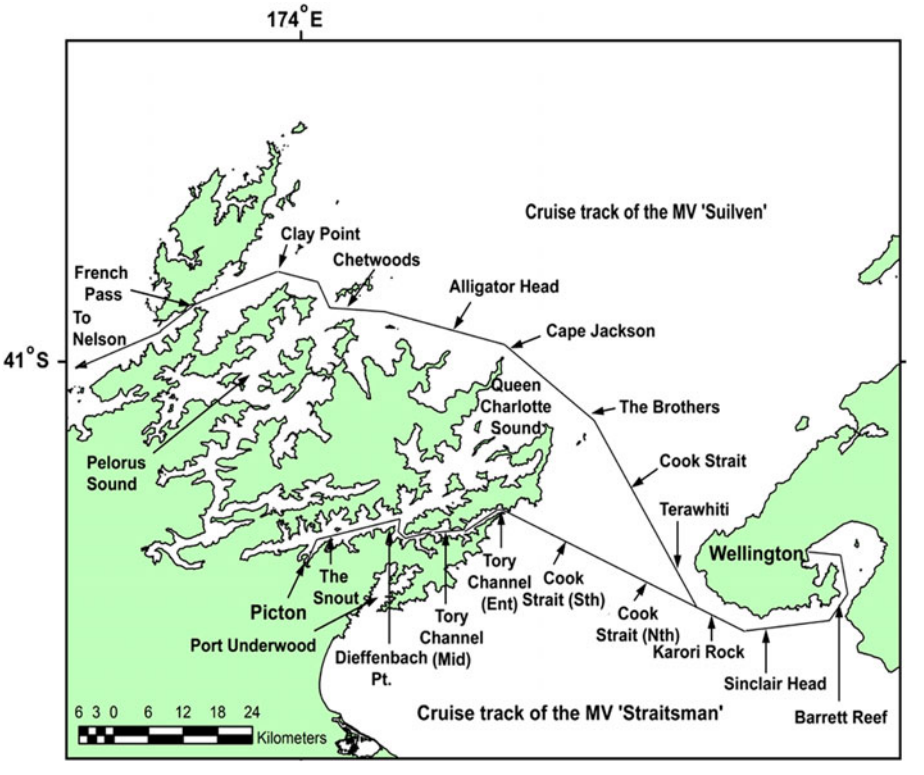

Figure 17 Stations sampled by the Cook Strait ferries MV *Suilven* and MV *Straitsman* every two to three days between September 2000 and March 2001.

found off Cape Jackson and the Brothers Islands on the western side of Cook Strait (Fig. 17; Table 3). Cell numbers peaked on 27 September 2000 with 2.0×10^4 cells/litre at Barrett Reef, 9.0×10^3 cells/litre at mid-Cook Strait and 5.0×10^3 cells/litre at the Brothers Islands. Lower numbers of cells were found on almost every sampling occasion (38) on the eastern side of the Strait from 13 September until 29 December 2000, after which no cells were observed. On a few occasions, cells were detected (4–40 cells/litre) in the western outer Sounds region, in the vicinity of the Chetwood Islands and French Pass. On five occasions (11, 13 and 20 October, 6 and 13 December 2000) cells were seen in samples to the west of D'Urville Island and French Pass in Tasman Bay. On 11 October 2000 low numbers of cells were observed in samples from Cape Soucis and off Nelson in Tasman Bay. In the samples collected from the MV *Suilven* on its voyages between Wellington and Picton (Fig. 17; Table 4), cells were frequently found in all samples from Wellington Harbour across the Strait and throughout the length of Tory Channel as far as Dieffenbach Point. Moderate cell numbers ($1.0–9.0 \times 10^3$ cells/litre) were sometimes seen in samples from mid-Cook Strait, though the usual pattern was for higher numbers nearer the North Island coast. A small number of cells were also seen in samples from inside Picton Harbour (1 and 3 November 2000).

On two occasions (16–18 August 2000 and 2–3 September 2000) sampling was carried out from the coastal container vessel, MV *Spirit of Resolution* (Pacifica Shipping Ltd). This vessel was transiting between the Port of Nelson and the Manukau Harbour (Onehunga), and between Onehunga and Lyttelton along the North Island west coast, through the Cook Strait and down the east coast

Table 3 *Gymnodinium catenatum* cell counts in selected samples collected by the MV *Straitsman* en route from Wellington to Nelson.

	27 Sept. 2000	11 Oct. 2000	10 Nov. 2000	20 Dec. 2000	22 Jan. 2001
Sample location	*Gymnodinium catenatum* cells/litre				
Barrett Reef	20,800	232	220	8	nd
Karori Rock	3600	2144	80	64	nd
Terawhiti	4		344		nd
Cook Strait	9600	336	792	nd	nd
The Brothers	5200	80	52	nd	nd
Cape Jackson	44	40	8	nd	nd
Alligator Head	nd	40	nd	nd	nd
Chetwoods	nd	nd	nd	nd	nd
Clay Point	nd	24	24	nd	nd
French Pass	nd	40	4	nd	nd
Cape Soucis	nd	8	nd	nd	nd
Pepin Island	nd	nd	nd	nd	nd
Nelson Boulder Bank	nd	12	nd	nd	nd

nd, not detected.

of the South Island. Samples were collected at one hour (c. 15 km) intervals for the duration of the voyages (20–32 h). These are the only data that give some idea of the offshore extent of the 2000–2001 *G. catenatum* bloom. On both occasions, the highest numbers of cells were observed on the Manukau Harbour bar (6.0–15.0 × 10^3 cells/litre), but there were also moderate numbers of cells (> 1.0 × 10^3 cells/litre) in surface waters at all sites sampled across the North Taranaki Bight, up to at least 70 km offshore (Fig. 13A,C). It is likely that cell numbers were much higher beneath the surface at these sites. These data shows there was an abundant and very widespread population of *G. catenatum*, extending well offshore north of Cape Egmont, that was more closely confined to the coast south of this point.

Table 4 *Gymnodinium catenatum* cell counts on selected dates from samples collected by the MV *Suilven* en route from Wellington to Picton.

	18 Sept. 2000	9 Oct. 2000	3 Nov. 2000	27 Dec. 2000	10 Jan. 2001
Sample location	*Gymnodinium catenatum* cells/litre				
Barrett Reef	44	808	880	8	nd
Sinclair Head	112	912			nd
Karori Rock	116	6824	404	24	nd
Cook Strait (north)	224	9200	760	1000	nd
Cook Strait (south)		688	44	300	nd
Tory Channel (entrance)	68	16	76	nd	nd
Tory Channel (mid)	68	8	nd	nd	nd
Dieffenbach Pt.	48	nd	4	nd	nd
The Snout	nd	nd	nd	nd	nd
Picton Harbour	nd	nd	28	nd	nd

nd, not detected.

Gymnodinium catenatum *cysts in seafloor sediments*

A survey of surface sediments at various locations, including the major ports, around the New Zealand coast was carried out between October 2000 and February 2001 (Table 5). This showed that *G. catenatum* cysts were widespread along the North Island west coast. High numbers of cysts were found in sediment samples collected offshore of Ninety Mile Beach, but none were detected in any samples collected from 10 intertidal sites. All sediment samples collected from the Kaipara Harbour contained cysts. Cysts were found at a number of sites in the Manukau Harbour, the iron sand loading terminal at Taharoa, and in Port Taranaki (New Plymouth). No cysts were found in any samples from the northeast coast of the North Island (Whangaroa Harbour, Bay of Islands, Mahurangi Harbour), although they were detected in a sample from Port Napier (Hawke Bay) and Wellington Harbour. No cysts were found at any South Island locations. Irwin et al. (2003) enumerated *G. catenatum* cysts and dated (^{210}Pb) sediment strata in cores from the Manukau, Hokianga and Wellington harbours to attempt to answer the question of whether this species had recently arrived in New Zealand or was indigenous. The results of their study demonstrated that *G. catenatum* had been present in the west coast harbours since at least the late 1930s, but that it was probably a new arrival in Wellington Harbour.

Table 5 The abundance of *G. catenatum* resting cysts in surface sediments throughout the North and South Islands, October 2000–February 2001 (data from Taylor & Mackenzie 2001).

Location	Date	*G. catenatum* cysts[a] (cyst/m^2)
North Island west coast		
Ninety Mile Beach (intertidal)	17/10/00	nd
Ninety Mile Beach (offshore)	17/10/00	10,200
Kaipara Harbour	28/02/01	2329
Manukau Harbour	05/12/00	860
Taharoa Iron-sand Terminal	12/01/01	460
Port Taranaki	07/12/00	2575
Wellington Harbour	08/12/00	7
North Island east coast		
Whangaroa Harbour	24/02/01	nd
Orongo Bay (Bay of Islands)	28/02/01	nd
Marsden Point	05/12/00	nd
Port Whangarei	06/12/00	nd
Mahurangi Harbour	27/02/01	nd
Waitemata Harbour	04/12/00	nd
Coromandel	08/10/00	nd
Tauranga Harbour	03/12/00	nd
Port Napier	02/12/00	13
South Island		
Marlborough Sounds	24/11/00	nd
Picton Harbour	24/11/00	nd
Nelson Haven	05/10/00	nd
Westport	14/12/00	nd
Timaru Harbour	20/01/01	nd
Lyttelton Harbour	19/01/01	nd
Port Chalmers	21/01/01	nd
Bluff Harbour	25/01/01	nd
Stewart Island	23/01/01	nd

nd, not detected.
[a]Mean of values from three sample sites at each location.

Gymnodinium catenatum risk assessment

Based on a knowledge of the distribution of motile cells and resting cysts of *G. catenatum* in 2000–2003, an assessment of the risk of blooms in the major shellfish growing areas of the country was made (Tables 6–7). There is a high risk that blooms will again develop on the North Island west coast and affect shellfish aquaculture in this region (Table 6). Also, because this region is still the major source of juveniles for other areas (Marlborough, Coromandel, Stewart Island), blooms in this region present a risk to other aquaculture zones that may not be directly affected. Apart from the Marlborough Sounds, the risk of the natural appearance of *G. catenatum* in South Island waters is probably low (Table 7).

Gymnodinium catenatum cysts in 'Kaitaia spat'

The New Zealand mussel industry is critically dependent on natural spat falls on drift algae cast

Table 6 The risk of the natural development of *G. catenatum* blooms in North Island shellfish growing areas.

Location	Risk	Comments
Kaipara Harbour, Hokianga Harbour	High	High numbers of cells, cysts and levels of PSP-toxin observed during 2000 and 2003 blooms.
Waikato west coast estuaries (Raglan, Kawhia, Aotea)	High	High numbers of cells, cysts and levels of PSP-toxin observed during 2000 and 2003 blooms.
Coromandel	Moderate	No evidence of *G. catenatum* or any PSP-toxin positive samples have been observed. There is the possibility of its transport into the area from the Bay of Plenty. Introduction with oyster and mussel spat possible.
Bay of Plenty	Moderate/ high	Low numbers of cells and low levels of PSP-toxin observed during 2003 bloom but not seen since, history of PSP contamination due to *Alexandrium* spp. blooms.
Hawke Bay	Moderate/ high	Recurrent blooms (April–July) with high cell numbers and levels of toxicity in 2000, 2001. 2002, 2003. Not seen since 2007.
Auckland-Northland East coast	Moderate	There have been no observations of *G. catenatum* from the Hauraki Gulf northwards. The southward flowing East Auckland Current may prevent natural introductions from further south. Introduction on oyster and mussel spat possible.

Table 7 The risk of the natural development of *G. catenatum* blooms in South Island mussel growing areas.

Location	Risk	Comments
Port Underwood	Moderate	Cells and cysts in the inlet during the 2000 bloom but no evidence of long term establishment. Reoccurrence of the 2000 bloom intensity and distribution (i.e. entry into Cook Strait) may lead to a reintroduction.
Outer sounds (East Bay, Tory Channel, Port Gore, Admiralty Bay)	Moderate	Low numbers of cells and cysts in outer sounds during 2000–2001. Particularly persistent in Port Gore/Queen Charlotte/Tory Channel but no evidence to date of long term establishment.
Pelorus and Kenepuru Sounds	Low/ moderate	Cells were often present in waters near Pelorus Sound entrance in 2000 but there was no evidence of these being introduced into enclosed waters of the sounds. Establishment of resident plankton/cyst populations in inner sound waters could lead to a chronic problem with recurrent blooms.
Tasman and Golden Bays (including Croisilles Hbr)	Low/ moderate	Introduction of cells into Tasman Bay through French Pass occurred in 2000 though there was no evidence of long term establishment.
Banks Peninsula	Negligible	No evidence of *G. catenatum* in South Island waters south of Port Underwood and northward flowing currents on South Island east coast mean contamination from the north is unlikely.
Stewart Island	Negligible	Natural arrival unlikely. Cooler waters in Stewart Island are probably within the range of tolerance of *G. catenatum* and transfer on mussel spat is possible.

up on Ninety Mile Beach (Northland) for around 80% of its seed. For many years, this material, colloquially known as 'Kaitaia spat', has provided a cheap and convenient source of juveniles, even if at times the supply has been somewhat erratic (Alfaro et al. 2004). The potential biosecurity risk of transferring toxic dinoflagellate cysts first arose in the early 1990s when cysts of *Alexandrium ostenfeldii* were discovered in Kaitaia spat (MacKenzie & Kappa 1992). As a result of these findings, and the discovery of PST in surf clams from Ninety Mile Beach in 1993, methods of minimising this risk were investigated (Hayden 1993; Hickman et al. 1994; Hay et al. 1994). In samples of Kaitaia spat collected in June 2000, low numbers (89 cysts/kg weed) of *G. catenatum* resting cysts were first observed and the industry immediately imposed a voluntary ban on its transport and use. This occurred during a period when mussel farmers would normally be expecting the best spat falls of the year and were preparing to restock their farms. Subsequent examinations revealed very high numbers of cysts (> 40,000 cysts/kg of weed) on some batches of Kaitaia spat and it became obvious that the ban on the use of this material would be long term and could seriously affect the future production and viability of the mussel industry. In response, the New Zealand Mussel Industry Council Ltd (2001) developed a code of practice designed to control mussel spat movement and therefore prevent the transfer of *G. catenatum* to other parts of the country. The protocols that were developed outlined the monitoring requirements, closure and opening criteria, procedures and responsibilities associated with spat movements between affected and non-affected areas.

For the estimation of the number of cysts in the seaweed containing the mussel spat (Kaitaia spat), the protocol described by Todd (1999) was used. Essentially, this involved thoroughly washing cysts off subsamples of the weed with high pressure water jets, trapping the cysts on screens and counting by microscopy. A practical detection limit for lot testing of Kaitaia spat was set at 100 cysts/kg.

Between June 2000 and January 2001 some very high cyst numbers (a maximum of 40,534 cysts/kg on 10 August 2000) were encountered (Fig. 18). After April 2001 (apart from one sample on 5 June 2001 of 124 cysts/kg) all batches returned nil results (New Zealand Mussel Industry Council Ltd 2001). Based on the first risk assessment a conservative opening criteria of 25 clear samples (i.e. containing no cysts) of Kaitaia spat recorded over a period of six weeks was in operation at this time. In July 2002, an analysis of the accumulated data showed that although occasional cysts were detectable in spat samples these were always below 100 cysts/kg, there were no increasing or decreasing trends and cysts were clearly at stable background levels. For this reason, the New Zealand Mussel Industry Council Ltd. (2002) amended the opening criteria to 10 samples taken over a period of no less than six weeks with <100 cysts/kg. Because the primary criteria (i.e. no sign of *G. catenatum* in Northland waters) and secondary criteria (i.e. no spat samples at > 100 cysts/kg for six weeks) had been met, the decision to open the beach was made on 26 July 2002. In hindsight, by applying the amended more liberal opening criteria, untreated or only primary treated (washed) Kaitaia spat could have been available from early August 2001 onwards. With the return of the *G. catenatum* bloom on the North Island west coast in 2003 (Fig. 16), Kaitaia spat was again contaminated with cysts and movement control procedures were activated. No further restrictions on the movement of Kaitaia spat have been necessary since, although it is probable that they will need to be invoked again sometime in the future.

As a consequence of the lack of Kaitaia spat, more effort was put into catching mussel spat in 'clean' areas, and there was an intense effort to develop methods of separating the mussel spat from the weed and the dinoflagellate cysts. When considering options for the treatment of Kaitaia spat it was decided in the early stages that attempts to selectively kill the cysts would be futile, although heat treatment remained a viable option for the treatment of oyster spat sticks. Alongside the various Kaitaia spat cleaning techniques that

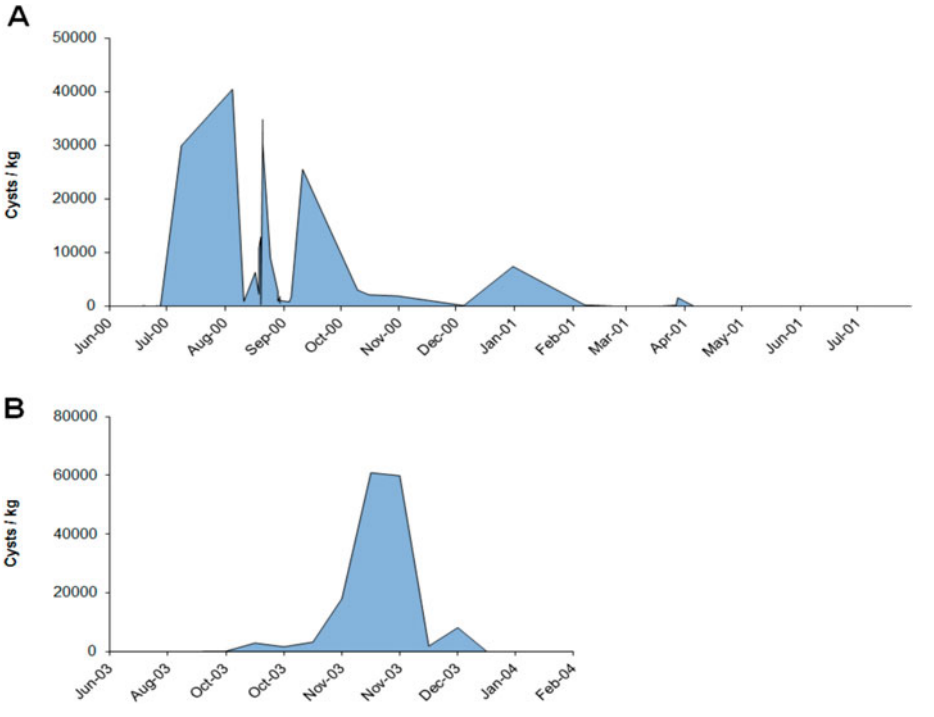

Figure 18 *Gymnodinium catenatum* cyst numbers (cysts/kg) in consignments of Kaitaia spat. **A**, Cyst counts 2000–2001; **B**, cyst counts 2003–2004.

were developed, a rigorous monitoring and quality control programme was put in place. Some decontamination methods were successful and several cleansing plants were established. In January 2001, the first cleaned mussel spat originating from Ninety Mile Beach was permitted to be placed in 'clean' growing areas of the Marlborough Sounds.

The North Island oyster industry was also affected as many of the best oyster spat catching areas are within the west coast estuaries, such as the Kaipara Harbour where *G. catenatum* was present. From here, spat is routinely transported to ongrowing areas on the east coast. In June, when high numbers of cysts were seen on oyster nursery sticks from the Kaipara Harbour, this transfer was immediately halted. Mussel spat catching lines in Aotea Harbour were also found to be contaminated with cysts.

Aside from effects on the molluscan shellfish industry, the bloom also affected the spiny lobster (*Jasus edwardsii*) fishery when it was found that PST with the characteristic toxin analogue profiles of *G. catenatum* were present in the digestive gland of the animals (Anon. 2001). Edible portions such as the tail flesh remained free of contamination, but because most product is exported as whole live animals, a precautionary ban was placed on this trade. After analyses showed that the toxin burden in whole crayfish was small, and having taken advice from overseas authorities with experience of PST in crustaceans, this ban was eventually lifted. A monitoring procedure has been developed by the New Zealand Rock Lobster Industry Council should it be necessary to again limit exports in the future.

The New Zealand and Australian abalone fisheries have also faced similar questions regarding the potential for PST contamination of wild abalone harvested during *Alexandrium* spp. and *G. catenatum* blooms. Low levels of PST have been found in the digestive gland of New Zealand pāua (*Haliotis iris*) during *G. catenatum* blooms

(MPI biotoxin data) and in the black-lipped abalone (*Haliotis rubra*) from areas off the eastern coast of Tasmania (Abalone Council Australia Ltd 2013). The risk to consumers is probably low, but a joint New Zealand and Australian industry research project is currently in progress to provide data that will assist in the future management of this issue.

Toxin profiles in New Zealand *Gymnodinium catenatum*

Cultured cells of New Zealand *G. catenatum* isolates predominately produce toxin analogues within the low potency N-sulfocarbamoyl group, mainly C3,4 and with smaller amounts of C1,2, GTX5 and GTX6 (Fig. 19). The more potent GTX1,4 analogues only make up a small proportion of the toxin but contribute substantially to the total toxicity. The toxin profiles of New Zealand *G. catenatum* isolates are very similar to those in the same species elsewhere in the world (Costa et al. 2014) and contain a comprehensive suite (Fig. 19B) of the low toxicity hydroxybenzoate analogues ('GC-toxins') that are unique to this species (Negri et al. 2003, 2007). Because of the lack of analytical standards for the GC-toxins these compounds cannot be accurately quantified as yet.

Effects on human health

There is no doubt that human poisoning can occur due to the consumption of shellfish contaminated with *G. catenatum* toxins (e.g. Rodrigues et al. 2012). However, it proved difficult for health agencies to accurately determine the effects of the

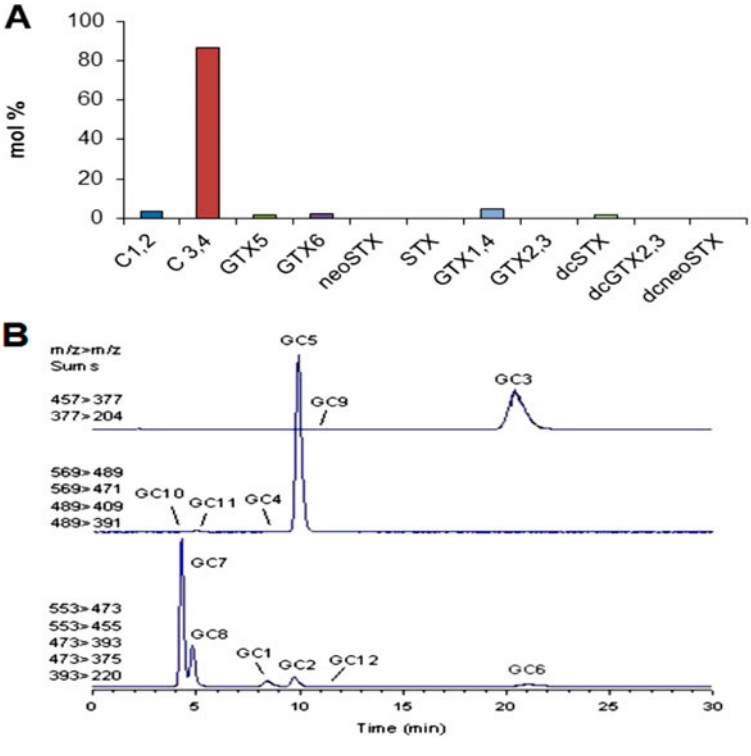

Figure 19 PST profiles in *G. catenatum*. **A**, Cultured cells of *G. catenatum* determined by pre-column oxidation HPLC (Manukau Harbour isolate; Cawthron culture collection ID: CAWD126); **B**, hydroxybenzoate saxitoxin analogues ('GC-toxins') determined by LC-MS (data courtesy of Dr Michael Quilliam, NRC, Canada) in a New Zealand isolate of *G. catenatum* (Cawthron culture collection ID: CAWD101).

2000–2003 blooms on public health because only a small number of cases meeting the case definition of 'PSP intoxication' were reported (Beauchamp 2000). In some areas, public health warnings were probably effective in preventing most people from gathering and eating shellfish. In other areas, such as Northland, where levels of toxicity have been highest and where shellfish are an important item of the diet, these warnings may have been ignored by some sections of the community. It was suspected (T. Beauchamp, Northland Health, pers. comm. November 2000) that there were unreported cases of mild poisoning, but had more serious cases occurred they would almost certainly have come to the attention of the public health system. The predominance of low specific toxicity N-sulfocarbamoyl toxins in the algae and shellfish may explain the few reports of human illness. Because most of the areas affected by the *G. catenatum* blooms in 2000–2003 were outside the main commercial areas, most of the financial burden for the extra monitoring was borne by central government via the Ministry of Health (MOH). The MOH commissioned a review of the options (Hay et al. 2000) to rationalise the existing monitoring programme in an effort to reduce these costs. It looked at retaining a viable system that still minimised the risk of poisoning to consumers of non-commercial shellfish. This report identified key primary sampling sites based on an analysis of data collected during the bloom and made recommendations that reduced the number of sampling sites and the frequency of sampling, and substituted phytoplankton monitoring for flesh testing at a number of sites. As a consequence of these cut backs, the detailed extensive spatial coverage that provided a near real-time picture of the spread of the bloom in 2000–2001 will probably not be available when there is a repetition of such a large-scale event in the future.

Conclusions

The organisms responsible for producing PSP in New Zealand are the same species (*Gymnodinium catenatum*, *Alexandrium catenella/tamarense* and *Alexandrium minutum*) that are notorious in temperate environments elsewhere in the world. Contamination by PST has not been a major impediment to shellfish aquaculture in New Zealand, but there have been several bloom events that have had important economic effects. Large-scale aquaculture developments in new areas with known histories of PST contamination, such as the Bay of Plenty and Hawke Bay, will need to work around this problem. There is also the possibility that *A. catenella* may become a chronic problem in the Top of the South region in the future, if it extends its range beyond QCS. The prohibition on the relocation of Kaitaia spat during the 2000–2003 *G. catenatum* blooms had the greatest effect on the industry as a whole. It is probable that a similar event will reoccur at some time in the future and the industry needs to be prepared for this. New biotoxin phenomena, such as the appearance of tetrodotoxin (TTX) in sea slugs in Auckland in 2010 (McNabb et al. 2010), occur in New Zealand from time-to-time. In this case the precise biosynthetic origin of the toxin is still unknown. There is no such uncertainty with regard to the PSP-causing toxins. The dinoflagellates that produce the toxins are well known and we now know how spatially extensive or constrained blooms can be in specific locations and, in general, how these will play out over time. We are developing a good idea of the major biological and environmental drivers of blooms in some locations (e.g. Opua Bay), but our knowledge is inadequate in other important areas (e.g. Bay of Plenty). There is generally a good relationship between cell numbers in the plankton and levels of toxicity in shellfish if sampling is carried out rigorously at appropriate locations. Phytoplankton monitoring usually gives ample early warning of the appearance of toxicity in shellfish. The cessation of MBA testing for PST in 2010 was an important step forward for the industry as this was becoming increasingly untenable on animal welfare grounds. Technical improvements to test methods (e.g. the adoption of mass spectrometric analysis and molecular methods for cell and cyst enumeration) can be expected in the future, which will make these even more robust, accurate and cost effective than they are at present. The recent serious PSP incident in the Bay of Plenty, and the Tasmanian shellfish industry's experience with the export of

contaminated products, are reminders that toxic phytoplankton and shellfish biotoxin monitoring is essential for quality assurance and the future of shellfish growing in New Zealand.

Acknowledgements

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