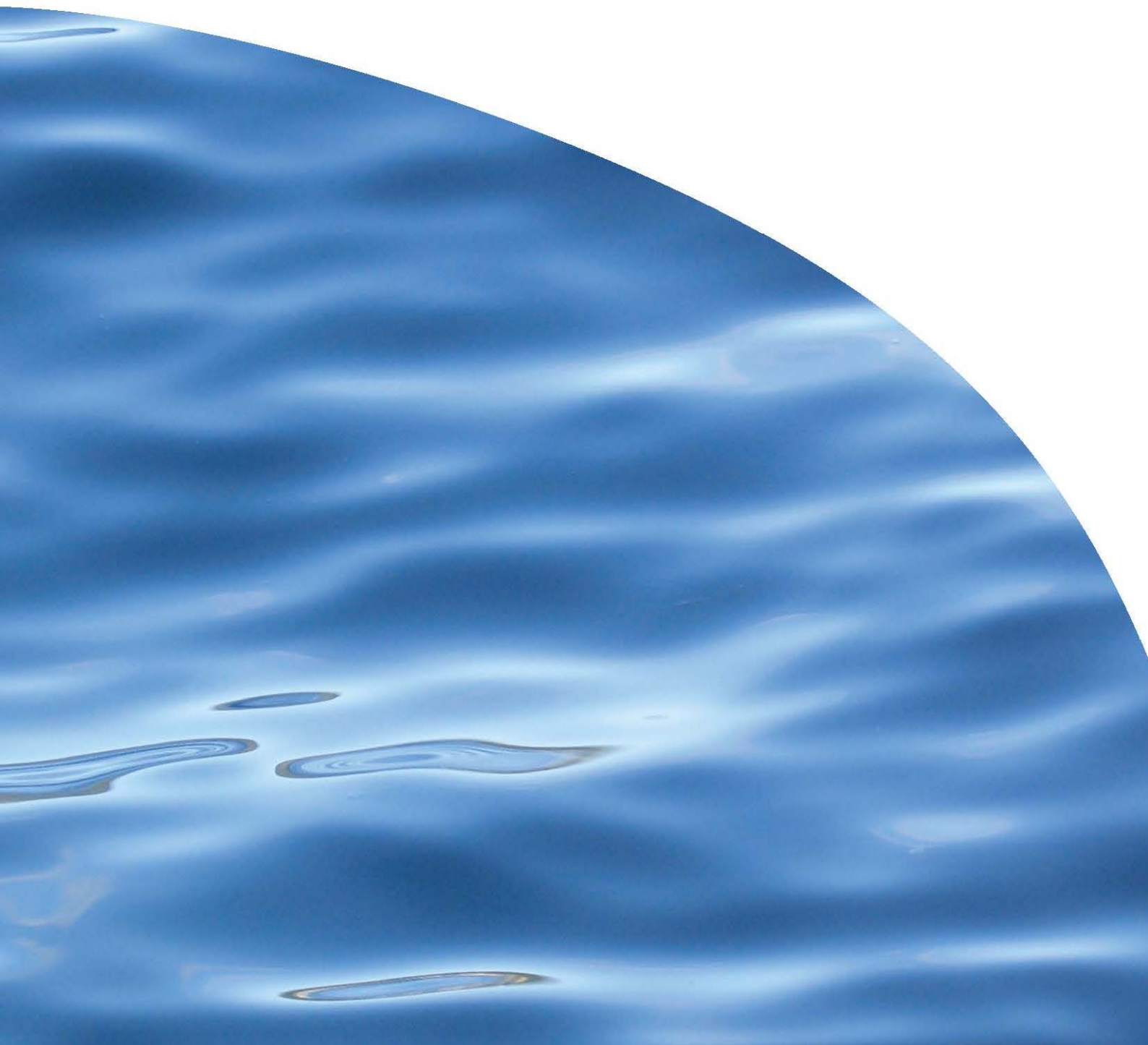


REPORT NO. 2346

**SEAFOOD SAFETY RISKS FROM PARALYTIC
SHELLFISH POISONING DINOFLAGELLATE
BLOOMS IN NEW ZEALAND: 2012-2013**



SEAFOOD SAFETY RISKS FROM PARALYTIC SHELLFISH POISONING DINOFLAGELLATE BLOOMS IN NEW ZEALAND: 2012-2013

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Prepared for the Ministry for Primary Industries, Food Safety


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EXECUTIVE SUMMARY

- An intense bloom of *A. catenella* developed in Opuia Bay, Queen Charlotte Sound during the 2013 summer. The bloom began in January, reached a climax in mid-March, and rapidly declined in early April.
- At the height of the bloom cell concentrations exceeding 1×10^6 cells/L existed in the Bay, causing extensive areas of visible 'red tide'.
- Monitoring the Opuia Bay dinoflagellate community provided a good early warning (> 1 month) of the appearance of toxicity in shellfish in adjacent areas of Tory Channel. It is recommended that routine sampling is carried out at this site between January and April each year.
- In contrast to the same period in 2012, February and March 2013 in Marlborough was exceptionally fine and sunny. It is believed that this created the conditions (high light and a stable stratified water column) that were conducive to the proliferation of the bloom. Importantly, the strong south-easterly gales which disrupted the development of the bloom in March 2012 did not occur in 2013.
- Monthly sampling of water column properties along a transect from Tory Channel to the head of Opuia Bay provided data on the conditions associated with the development of the bloom. The pre-existing cyst bed, the hydrodynamic characteristics of the Inlet, seasonal thermal stratification, and the supply of nutrients from the deeply mixed, naturally nutrient enriched environment of Tory Channel are the main factors which drive the annual *A. catenella* bloom. An *in situ* net population doubling time of ~8.3 days was estimated.
- Large swarms of the lobster krill *Munida gregaria* existed over the bloom period in Tory Channel and the Onepua/Opuia Inlet and were found to be accumulating paralytic shellfish poisoning-toxins (PSP-toxins). An experiment showed that *M. gregaria* can reduce *A. catenella* cell numbers in the water column and it is possible it had some impact of the progression of the bloom. *M. gregaria* provides a route for food chain transmission of PSP-toxins to higher trophic levels.
- The PSP-toxicities of shellfish throughout the Sound in 2013 were substantially lower than in 2011 and the closure period was consequently shorter. Toxicity at sites affected by Tory Channel water flows were probably contaminated due to the export of cells from the Onepua/Opuia Inlet. However, in the case of East Bay, it is likely that the bloom there developed independently, originating from cyst beds in this area.
- Low numbers of *A. catenella* were observed in Port Underwood and Port Gore in late March early April.

- A new analysis of *A. catenella* resting distribution in SCUBA retrieved sediment cores from Opua Bay, found apparently viable cysts at a depth of at least 20 cm. This suggests that the dinoflagellate has been resident in the Bay for multiple decades. Isotope dating of sediment strata is in progress to assign dates to sedimentary layers.
- In December 2012 the most serious PSP poisoning event documented in NZ to date occurred, due to people consuming contaminated Tuatua from ocean beaches in the vicinity of Tauranga. At least 29 people became ill, several seriously, despite the fact that a public health warning was in place.
- Affected shellfish associated with cases of human poisoning had high levels of toxicity (31 mg STX equiv'/kg and 14 mg STX equiv'/kg on the Lawrence HPLC screen and confirmation tests respectively) with toxin profiles dominated by the most toxic PSP-toxin analogues, STX and neoSTX.
- The cause of the event was a bloom of *Alexandrium minutum*, a species which has been implicated in PSP-toxin contamination in this area a number of times previously. Phytoplankton monitoring at the Tauranga and Bowentown sites provided an early warning of the appearance of *Alexandrium minutum*.
- Historically, *A. catenella* has been the main cause of PSP-toxin contamination in the Bay of Plenty (BOP) but the toxin profiles of *A. catenella* and *A. minutum* are qualitatively different. The former has a profile containing a large proportion of low toxicity N-sulfo carbamoyl analogues (C1,2, GTX5); the latter produces a substantial proportion of high toxicity STX and neoSTX analogues.
- It is assumed that scepticism by the public to toxic shellfish warnings in the BOP has arisen because of their high frequency and because the consumption of shellfish contaminated by *A. catenella* is less likely to cause illness than that by *A. minutum*. Public health warnings need to be able to distinguish between these different levels of hazard.
- In late winter and spring 2012 the toxic dinoflagellate *Gymnodinium catenatum* appeared in water samples, and low levels of PSP-toxins were detected in shellfish, at a number of public health monitoring sites on the North Island west coast. This species had not been observed in this area since it was last reported in 2007. The widespread and damaging bloom of this species that took place in 2000–2001 also developed in this region at this time of year. This suggests that a similar event, originating from the same area, could occur again.

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1. INTRODUCTION

Since the summer of 2011 it has been recognised there is a chronic problem with the occurrence of toxic *Alexandrium catenella* blooms in Queen Charlotte Sound (QCS), that sometimes result in widespread contamination of shellfish with paralytic shellfish poisoning-toxins (PSP-toxins) (MacKenzie *et al.* 2011, 2012). *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 Queen Charlotte Sound in 2010. *A. 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, Tasman and Golden Bays, the shellfish aquaculture industry may have to manage production around annual region-wide closures of 2–3 months in late summer and autumn.

A. 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 Opuia Bay off Tory Channel (Figure 1). Subsequent observations have shown that Opuia Bay is an important location where blooms are generated every year and from where cells are distributed to other areas under certain weather conditions. Opuia 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 Seafood Safety Programme (MBIE contract # CAWX0703), we have focused our research over the last two years on Opuia 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 in the future.

In the 2012 season (January–April 2012) a bloom of *A. catenella* began to develop in Opuia Bay and it appeared likely that there would be a repeat of the widespread closures of 2011. However the bloom was suppressed by strong south-easterly winds in late February and early March which lowered temperatures and disrupted water column stratification. From late March to early April the bloom re-developed, but competition from a co-occurring dinoflagellate and lateness of the season prevented it becoming dominant. No shellfish harvest closures were necessary that year.

In contrast to the 2012 summer, Marlborough experienced a warm, calm and very sunny period in February and March 2013. The highest sunshine hours ever recorded in Blenheim were experienced in February (since measurements began in 1947) and

above normal sunshine hours also occurred in March (NIWA Climate Summaries). Conversely the upper South Island experienced a cloudy April with the second lowest sunshine hours ever recorded in Blenheim during that month. The weather in March lead to the establishment of a stable stratified water column in Opuia Bay and the high light conditions favoured the luxuriant development of the *A. catenella* bloom.

In addition to the Queen Charlotte Sound *A. catenella* bloom, there were two other notable PSP-toxin events in other areas of New Zealand in 2012. In December 2012 a bloom of *Alexandrium minutum* in the Bay of Plenty (BOP) contaminated shellfish with high levels of saxitoxin (STX) and neo-saxitoxin (neo-STX) that led to the poisoning and hospitalisation of at least 29 individuals, despite a public health warning being in place at the time (see Section 9). In October 2012, after an absence for nearly 10 years, cells of the PSP-toxic dinoflagellate *Gymnodinium catenatum* were observed in water samples collected from various sites (Kaipara and Manukau harbours) on the North Island west coast, along with the appearance of toxicity in shellfish in the region. Fortunately the bloom did not develop in a big way. However it was a reminder that a major damaging event, like that which this dinoflagellate caused in the same area in 2001, could re-occur at any time.

This report presents a summary of recent research on the ecology of *A. catenella* in Queen Charlotte Sound and observations on the recent occurrence of other PSP-toxic blooms around New Zealand in 2012/13. The aim is to inform MPI on the nature and dynamics of these events and thereby help protect public health and the NZ shellfish industry.

2. OPUA BAY MONITORING

Phytoplankton samples from Opuia Bay were collected (Figure 2, Site 7) as part of the routine weekly shellfish and phytoplankton sampling programme by MSQP. Sampling usually took place on a Wednesday, between the hours of 10:00 and 13:00, and samples were collected from 3 m depth intervals (0 m, 3 m, 6 m, 9 m, 12 m, 15 m) using a van Dorn sampling bottle. Microscope counts of *A. catenella* cells and documentation of other species co-occurring with it in these samples were carried out. During the bloom period, from January to May 2013, reports were e-mailed to MSQP and affected shellfish farmers within 24 hours of the samples being collected. These reports contained a running tally of cell counts, graphical illustrations of trends (Figures 3 and 4), comments on the current situation and a tentative forecast of bloom progression.

Low numbers of *A. catenella* first appeared in the water column at Opuia Bay in October 2012 (Figures 3 and 4) and gradually increased in numbers through December and January. Over the week of 30 January–7 February there was a rapid increase in cell numbers with a dense layer of cells (3.5×10^5 cells/L) forming at 6 m depth. From then on cell numbers continued to increase, with the maintenance of a mid-water column concentrated layer, until it reached a climax during the week of 13 March. Over this period extensive ‘red tide’ patches were visible throughout the Bay (Figures 5 and 6) and cell numbers at the surface and in mid water column layers exceeded 1.0×10^6 cells/L. In early February MSQP were alerted that because of the rapidly increasing cell numbers, and the extended forecast for fine calm weather, that it was likely that a substantial bloom would develop this season.

Water column temperatures, recorded at the monitoring buoy in Opuia Bay, were higher in February and March 2013 than during the same period the previous year (Figures 7 and 8). More importantly, the strong south-easterly gales which broke down water column stratification and dispersed the bloom in March 2012 (Figures 9 and 10) did not occur in March 2013.

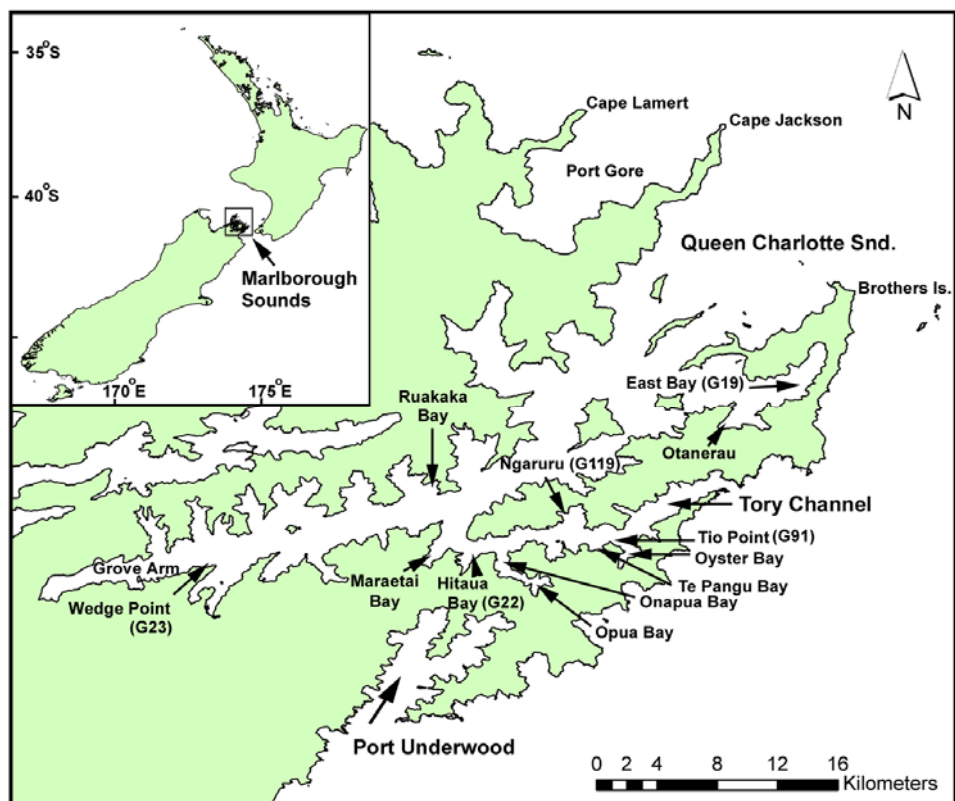


Figure 1. Locations in Queen Charlotte Sound (QCS) referred to in this report.

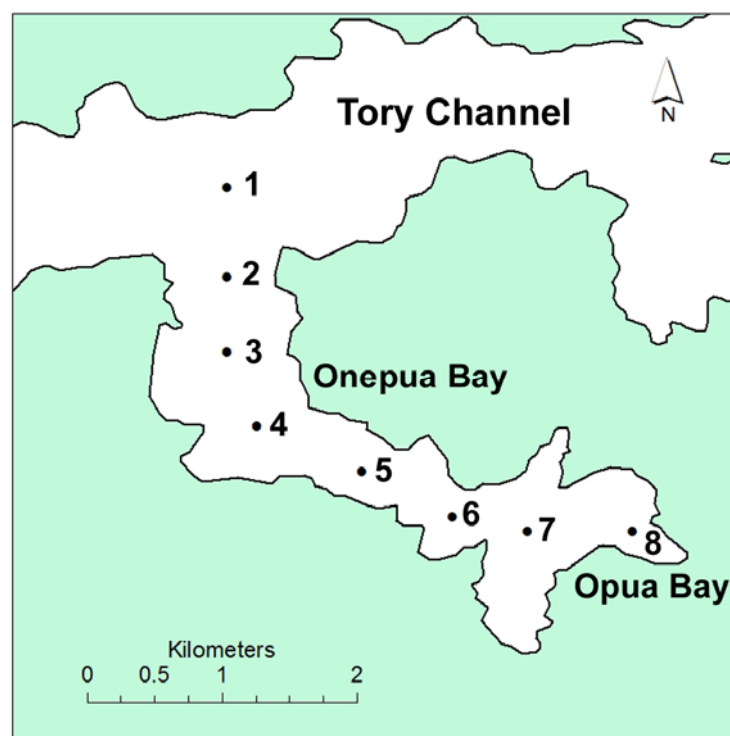


Figure 2. Sampling site locations on the Tory Channel–Opua Bay transect.

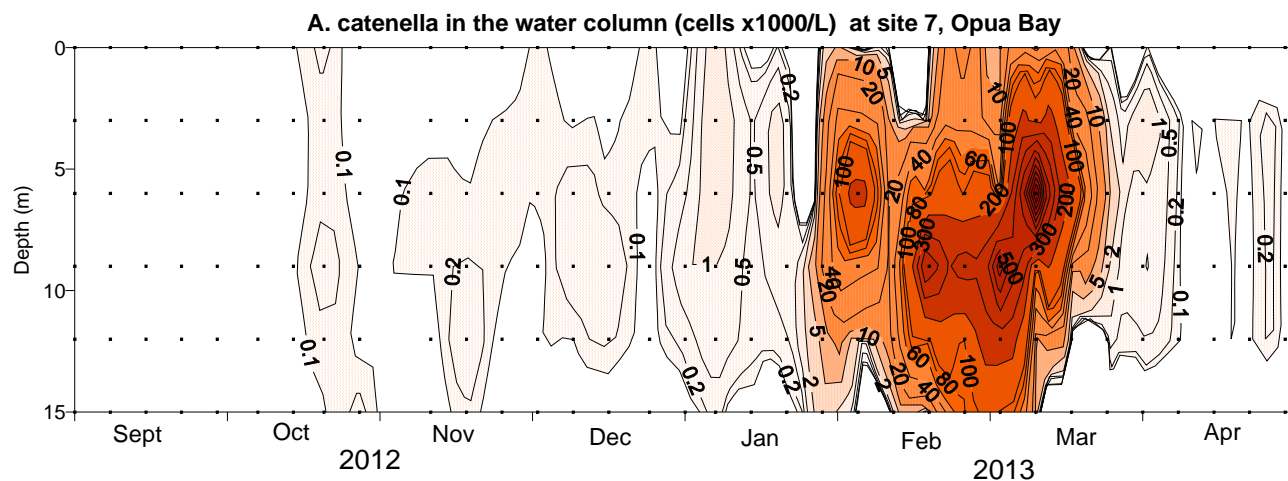


Figure 3. The distribution of *Alexandrium catenella* cell numbers in the water column at Site 7 Opua Bay September 2012–April 2013.

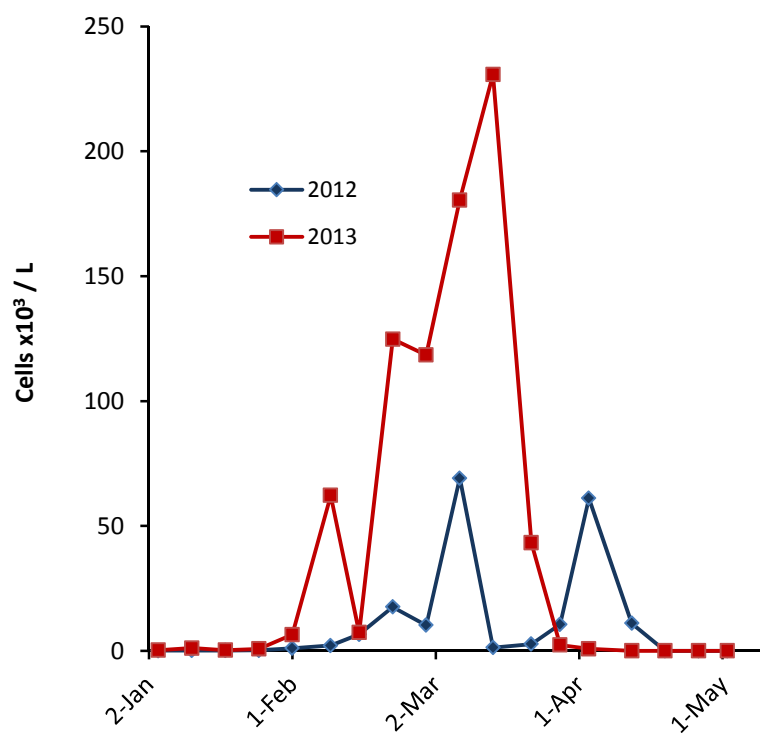


Figure 4. Depth averaged *Alexandrium catenella* cell numbers at Site 7 Opua Bay January–May 2012 and 2013.



Figure 5. View from hills above Opua Bay showing discoloration of surface waters due to the *Alexandrium catenella* bloom, 9 March 2013.



Figure 6. Harvesting bulk cell concentrates of *Alexandrium catenella* from surface aggregations. The monitoring buoy from which the temperature loggers were suspended is visible in the background.

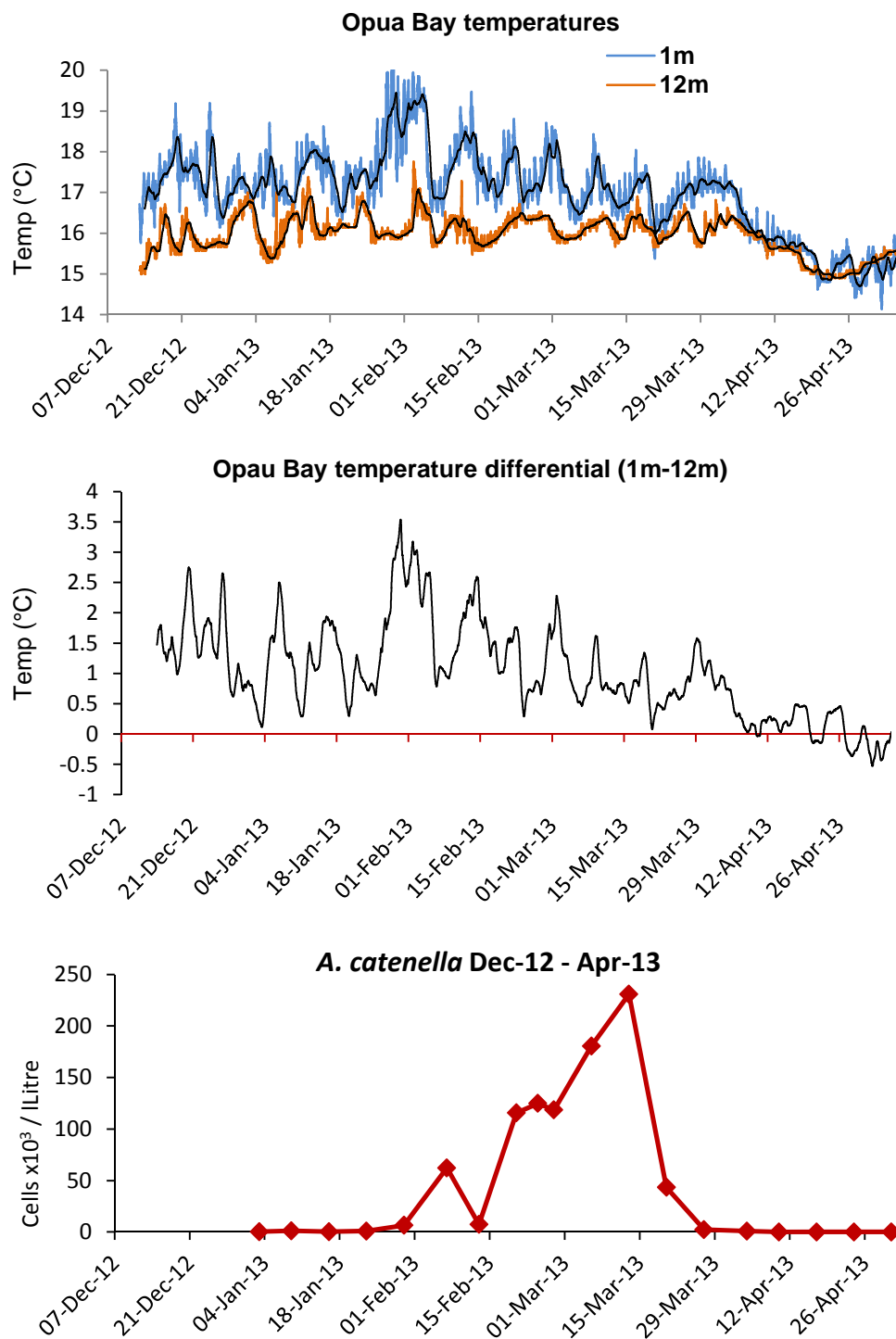


Figure 7. Near-surface and bottom temperatures at the Opau Bay monitoring buoy December 2012–April 2013.

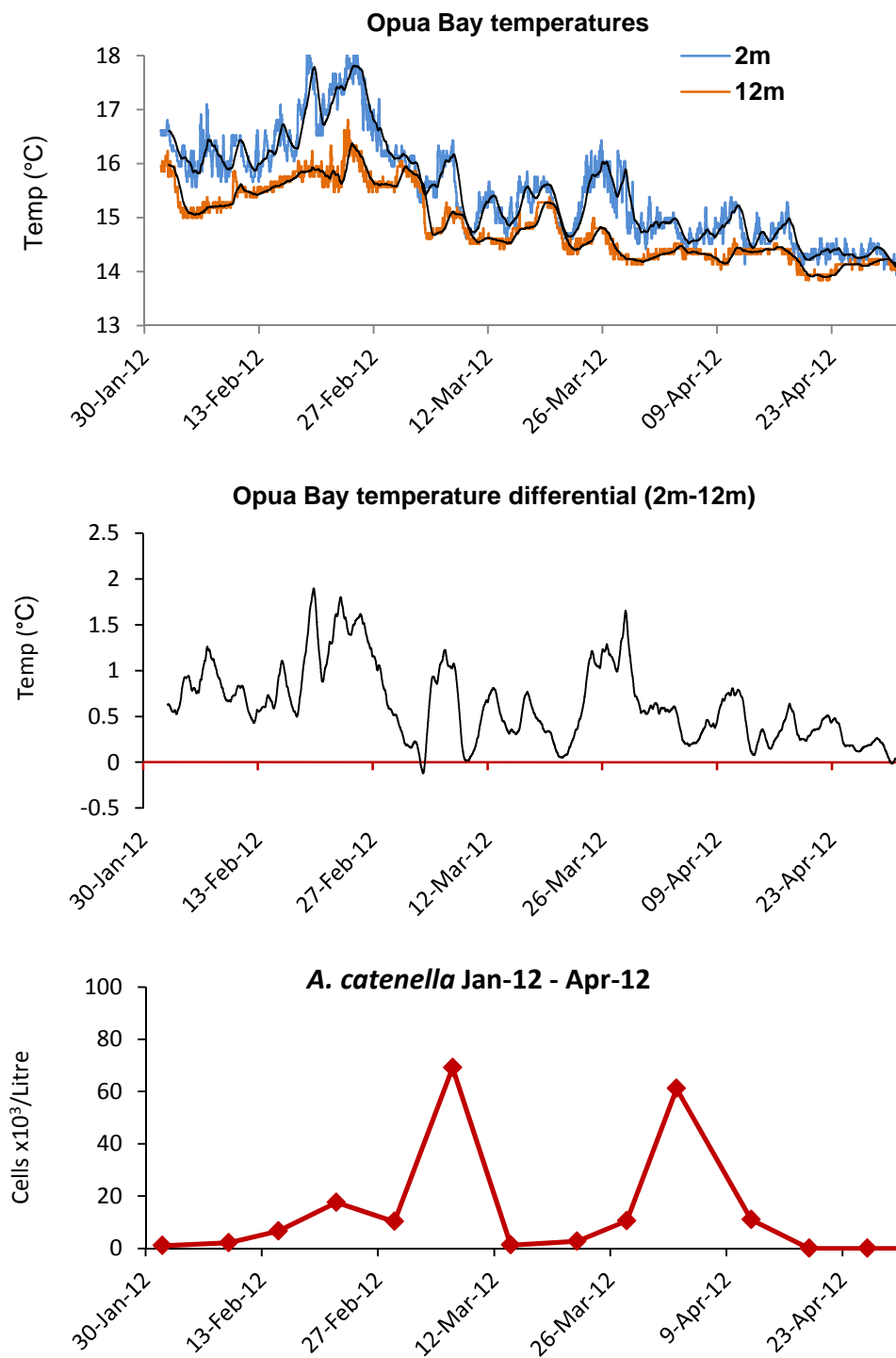


Figure 8. Near-surface and bottom temperatures at the Opua Bay monitoring buoy January–April 2012.

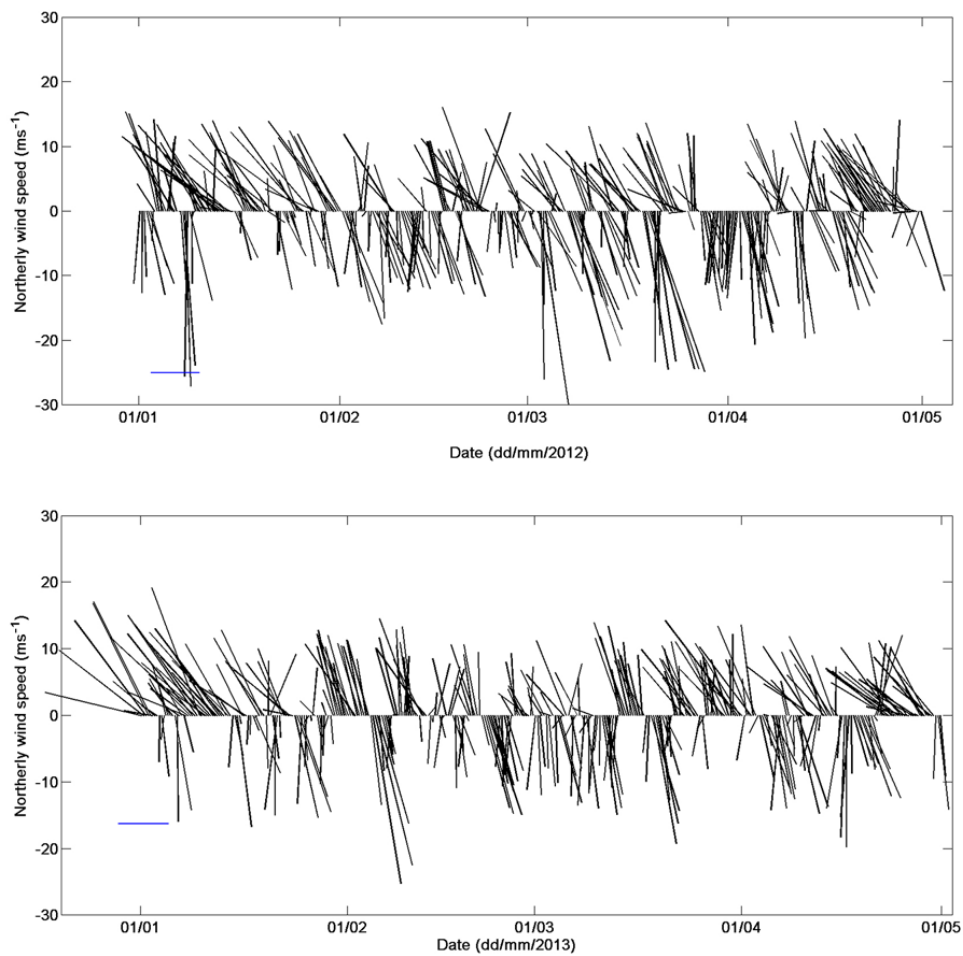


Figure 9. Vector plots of wind velocity and direction recorded at the Brothers Island weather station for the period 1 January–30 April 2012 and 2013.

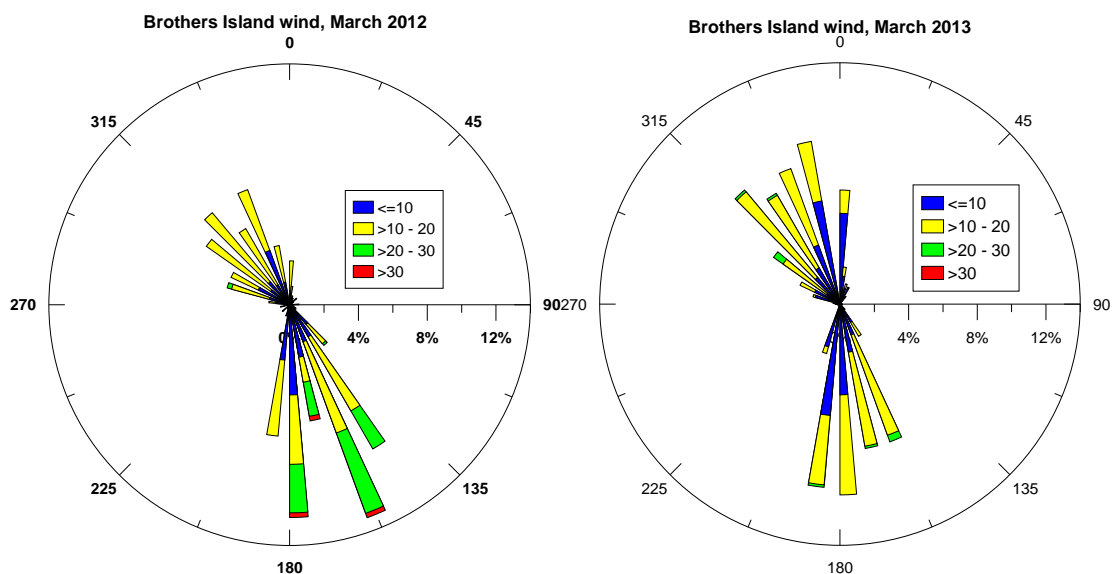


Figure 10. Wind rose diagrams showing the differences in wind velocity (cm/sec) and direction between March 2012 and March 2013.

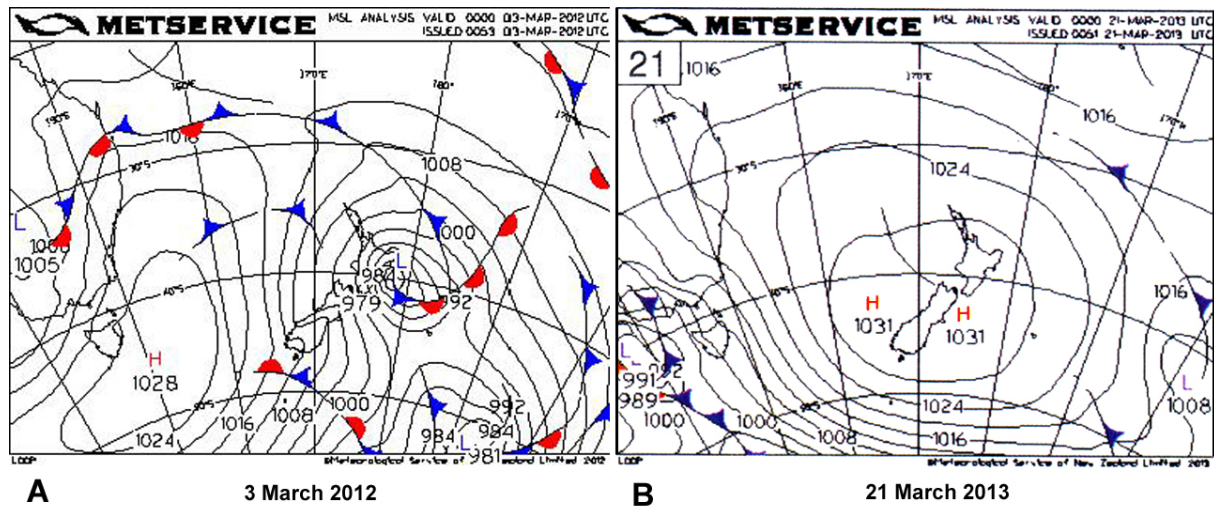


Figure 11. Tasman Sea midday isobar charts, 3 March 2012 and 21 March 2013, showing typical weather systems that directly affected the Marlborough Sounds in February and March of these years.

A series of low pressure systems from the northern Tasman Sea brought gale force southeasterly winds onto central New Zealand in February and March 2012 (Figure 11 A) that lowered water temperatures in Opua Bay and disrupted water column stratification. February and March 2013 was characterised by persistent, intense and slow moving anticyclonic systems (Figure 11 B) lying across central New Zealand, bringing fine calm weather for much of the period and exceptionally high sunshine hours in Marlborough.

3. TORY CHANNEL — OPUA BAY TRANSECTS

To obtain data on water column characteristics within the Onepua/Opua Inlet during the development of the *A. catenella* bloom, a transect from mid Tory Channel to the head of Opua Bay was sampled on four occasions at approximately 1 month intervals, between 12 December 2012 and 13 March 2013 (Figure 2). The observations have established baseline values essential for the parameterization of a biophysical bloom simulation model.

At each of eight stations along the transect CTD/fluorometer casts to measure salinity, temperature, light, turbidity and chlorophyll a fluorescence were made. Van Dorn water bottle samples at 3 metre depth intervals were also collected at each station for phytoplankton counts, dissolved inorganic nutrient, and extracted pigment analyses. On a number of other occasions 12 metre depth integrated samples were collected (with a Lund tube) from the same transect stations (Figure 12) for *A. catenella* cell counts. The data from the transect analyses are presented in Figures 12–16.

Table 1. *Alexandrium catenella* cells numbers at monitoring sites outside the Onepua/Opua Inlet.

<i>Alexandrium catenella</i> (cells/L)									
Queen Charlotte Sound						Port Underwood		Port Gore	
Sampling date	Hitaua Bay	Clay Point	Ruakaka Bay	Te Pangu Bay	Tio Point	East Bay	The Tongue	Opihi Bay	Melville Cove
13 Feb-13	100								
20 Feb-13	400								
22 Feb-13				200					
06 Mar-13	1200	400	400		600	200			
13 Mar-13	800		400			200			
17 Mar-13		4300		600					
18 Mar-13	1000	800	400	800		400			
20 Mar-13		600							
22 Mar-13		800							
26 Mar-13		400	3400						
27 Mar-13	1400		1000		900	1400		100	
4 Apr-13	800		4900	300	1700	2200	100		
8 Apr-13									300
Max	1400	4300	4900	800	1700	2200	100	100	300

Data courtesy of Marlborough Shellfish Quality Programme (MSQP) and NZ King Salmon Company Ltd.

These data clearly show that the *A. catenella* bloom developed and was mainly confined within the sheltered waters of the Inlet for much of the bloom period, especially within the inland reaches of Opua Bay (Figure 12). *A. catenella* cells that were observed in low numbers at various locations within Tory Channel (e.g. Hitaua Bay, Table 1) in February probably originated from the export of cells from the Onepua/Opua Inlet. Following the sudden decline in cell numbers in Opua Bay after 13–20 March, cell numbers at Tory Channel sites (e.g. Clay Point) rose substantially (Table 1) suggesting that mass transport of cells from the Inlet had occurred at that time. Because of high current flows, the Tory Channel water column is always deeply mixed, even at the height of summer and for this reason it is a habitat that is not conducive to the formation of flagellate dominated phytoplankton communities. The *A. catenella* cells that were observed at the Tory Channel sites are transient. At Tio Point (within Oyster Bay) and at Ruakaka Bay and East Bay (Figure 1) more stable water column conditions may allow *A. catenella* to become established, however at none of these locations in 2013 did these populations persist for long. Because of the distance between Opua Bay and East Bay (~20 km) it seems unlikely that transport of cells is responsible for the appearance of cells and toxicity in the mussels at this location. Surveys (MacKenzie *et al.* 2011) have shown that the sediments in East Bay contain a sizable population of *A. catenella* resting cysts and it is suspected that blooms may originate independently in this area. Future research will focus on attempting to establish the origin and dynamics of the *A. catenella* bloom in East Bay. *A. catenella* cells were observed (albeit at low numbers) in late March and early April in Port Gore and Port Underwood.

Due to restricted circulation in the Onepua/Opua Inlet, surface waters become warmer than the main channel in summer. There is little variation in salinity with depth in Tory Channel or within the Inlet, and temperature is the main determinant of water column stratification (Figures 13–16). Unusually for sea waters in the Marlborough Sounds nitrate-N concentrations in Tory Channel remain high within the entire water column ($>3 \mu\text{M}$) throughout the summer, due to the continuous input of oceanic water from Cook Strait (Figures 13–16). It is believed that this is the most important factor that leads to the high phytoplankton biomass in the various embayment's that extend off the main channel, such as the Onepua/Opua Inlet. The very high biomass of *A. catenella* that developed during February and March resulted in the complete depletion of measurable nitrate-N throughout much of the water column in the inner part of the Inlet (Figures 14 and 15). Although there was some apparent decrease in dissolved reactive phosphorus (DRP) concentrations associated with the bloom in Opua Bay (Figure 16) there was no evidence of depletion.

On one occasion, soon after heavy rainfall, high nitrate-N concentrations ($6\text{--}7 \mu\text{M}$) at the surface in Opua Bay were observed associated with a low salinity layer emanating from the small creek at the head of the Bay (Figure 14). The *Pinus radiata* forest within catchment of Opua Bay has been clear-felled within the last few years and runoff is minimally impeded in its present condition. This may be this cause of the

transient nutrient enrichment episode. In comparison to the ambient concentrations of nitrate present in the seawater entering the inlet from Tory Channel the freshwater contribution is probably insignificant.

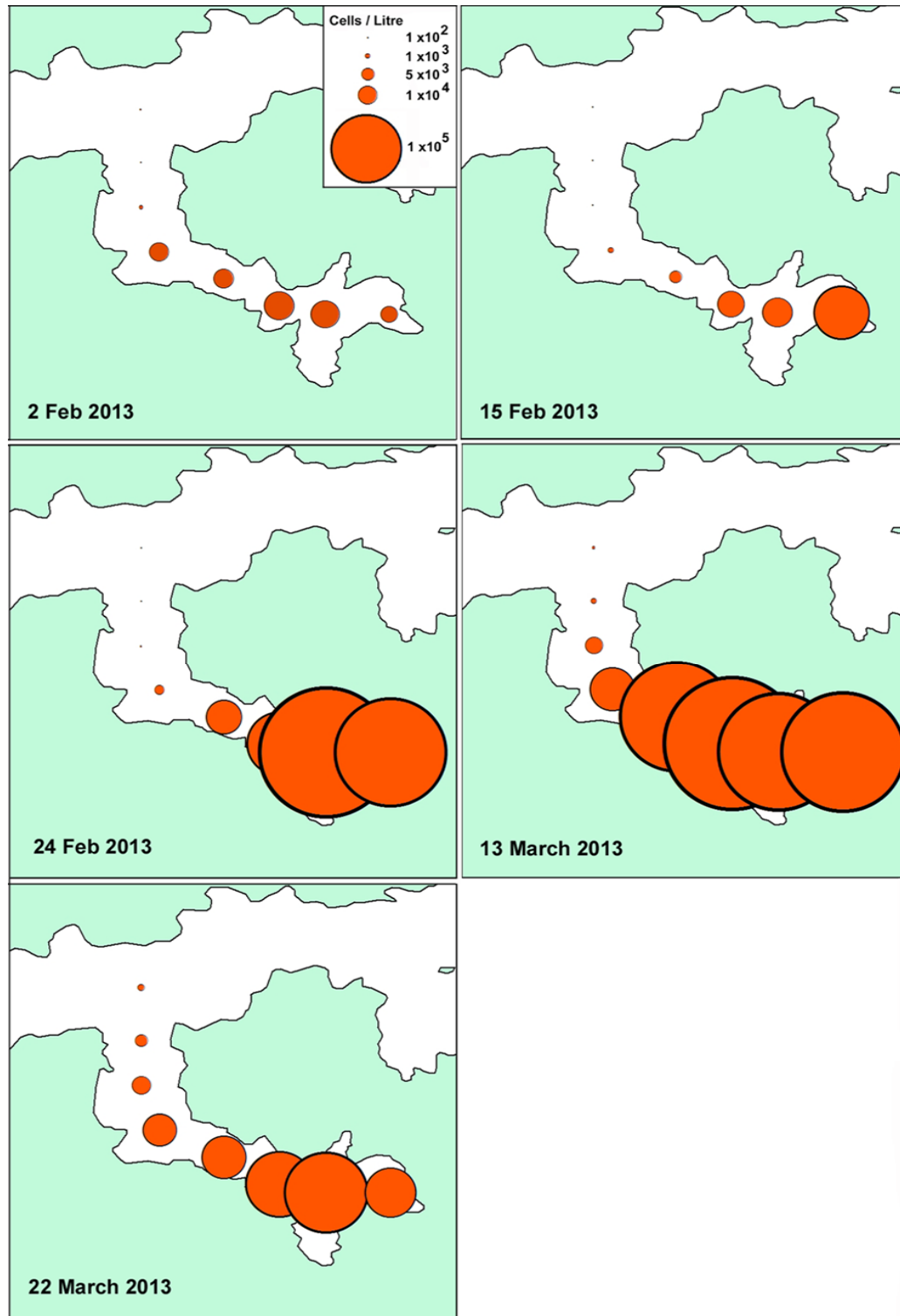


Figure 12. Cell counts of 12 metre integrated water column samples, showing the development of the *Alexandrium catenella* bloom in the Onepua /Opua Bay Inlet February-March 2013.

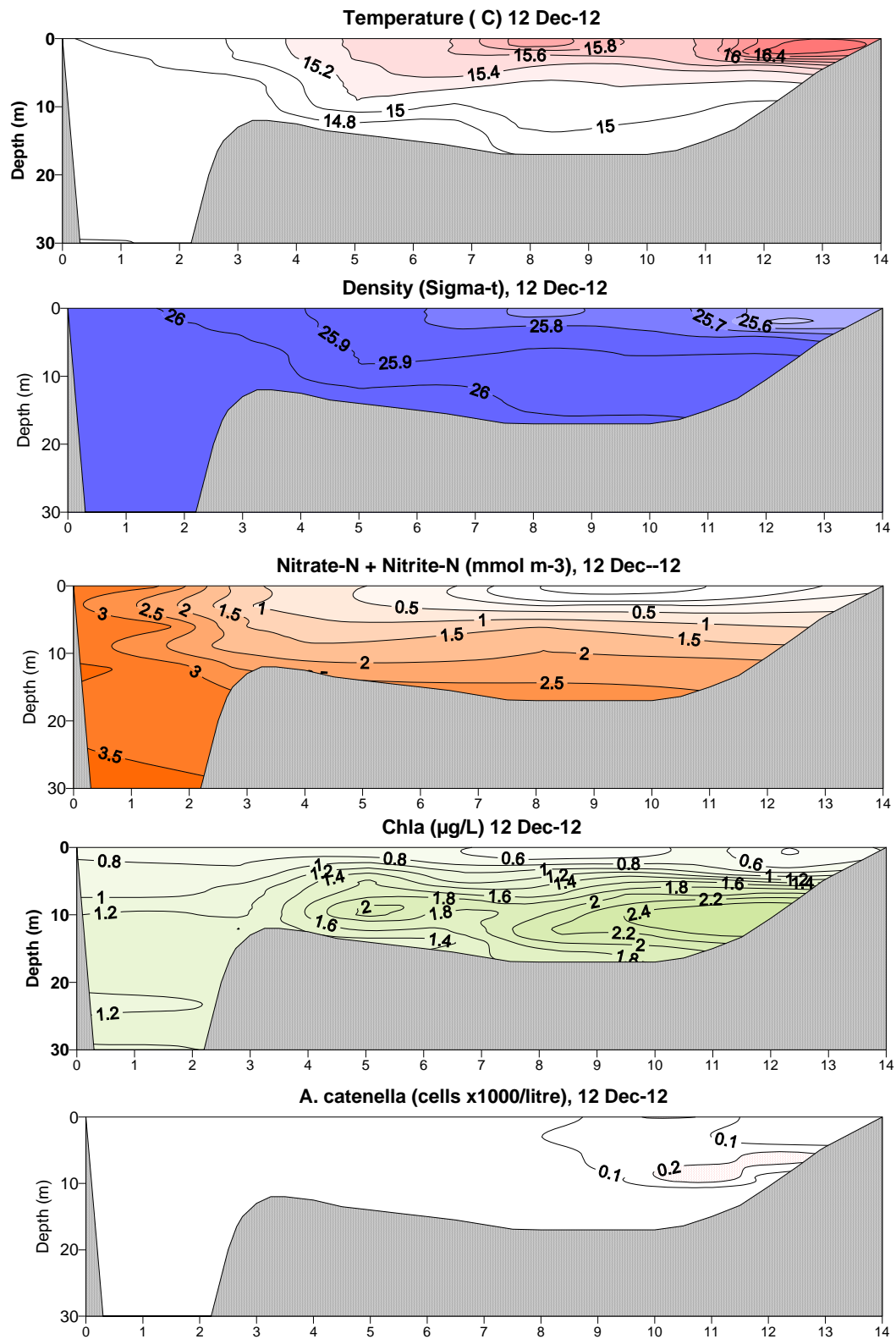


Figure 13. Tory Channel–Opua Bay transect sampled 12 December 2012.

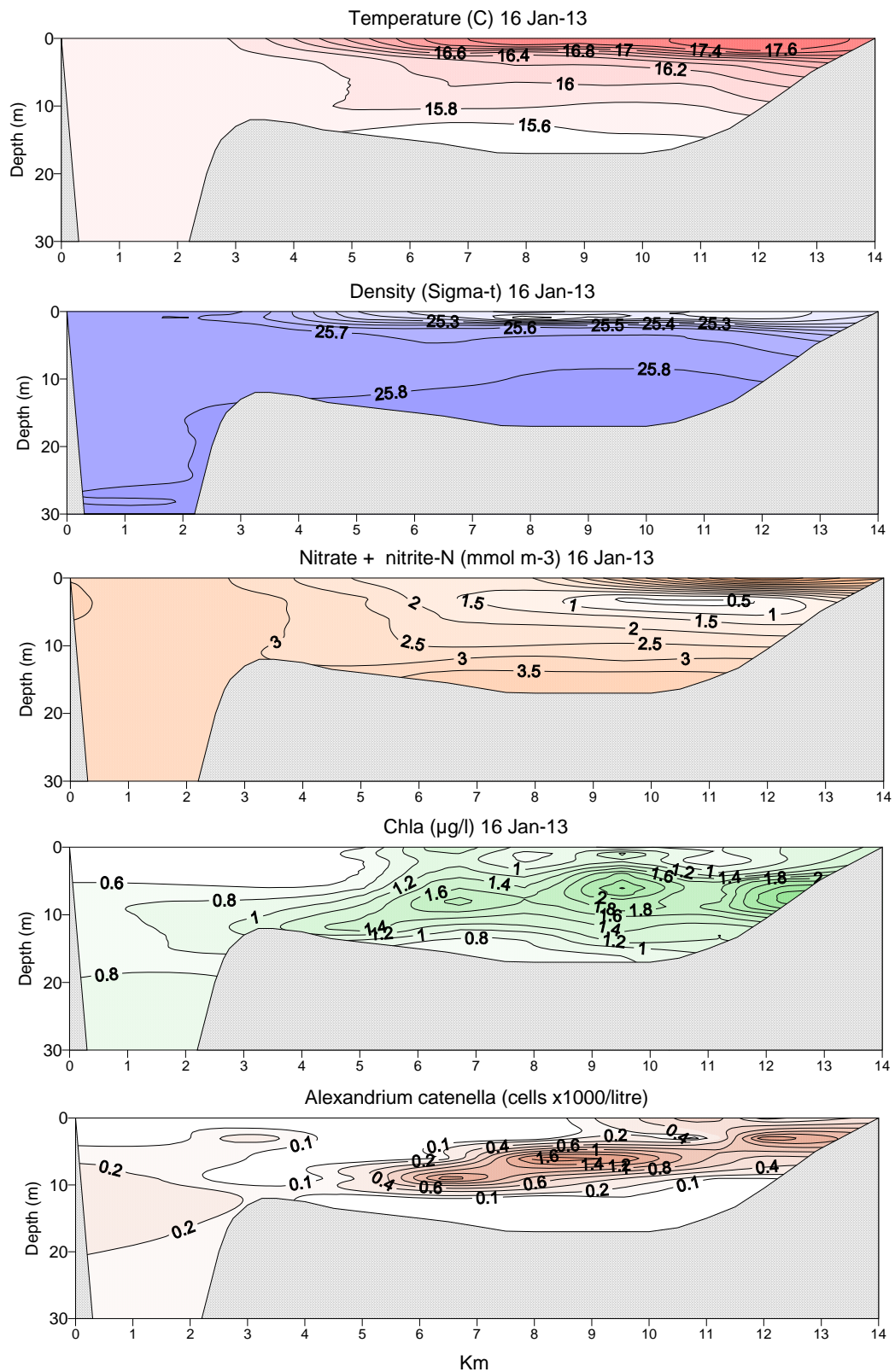


Figure 14. Tory Channel–Opua Bay transect sampled 16 January 2013.

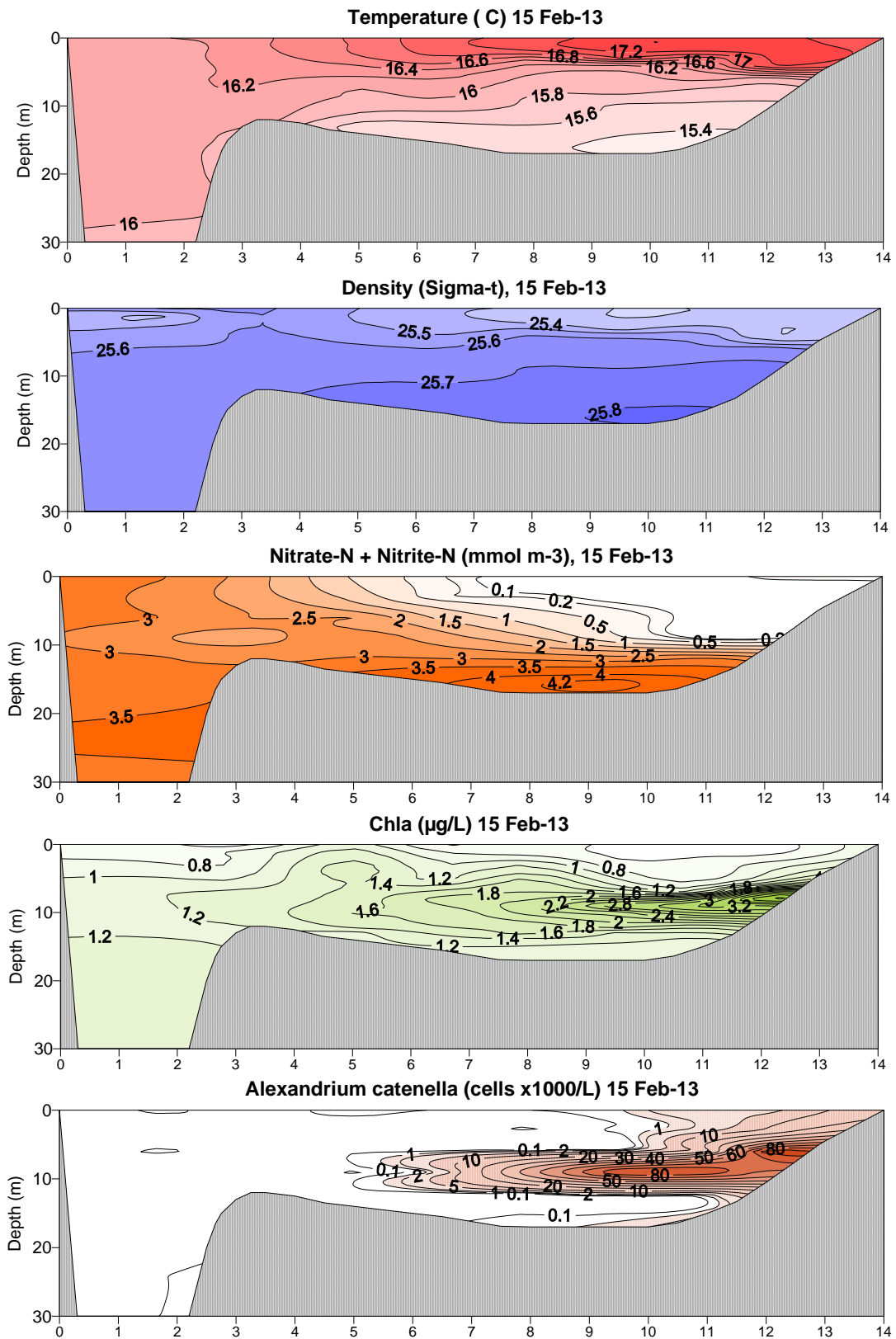


Figure 15. Tory Channel–Opua Bay transect sampled 15 February 2013.

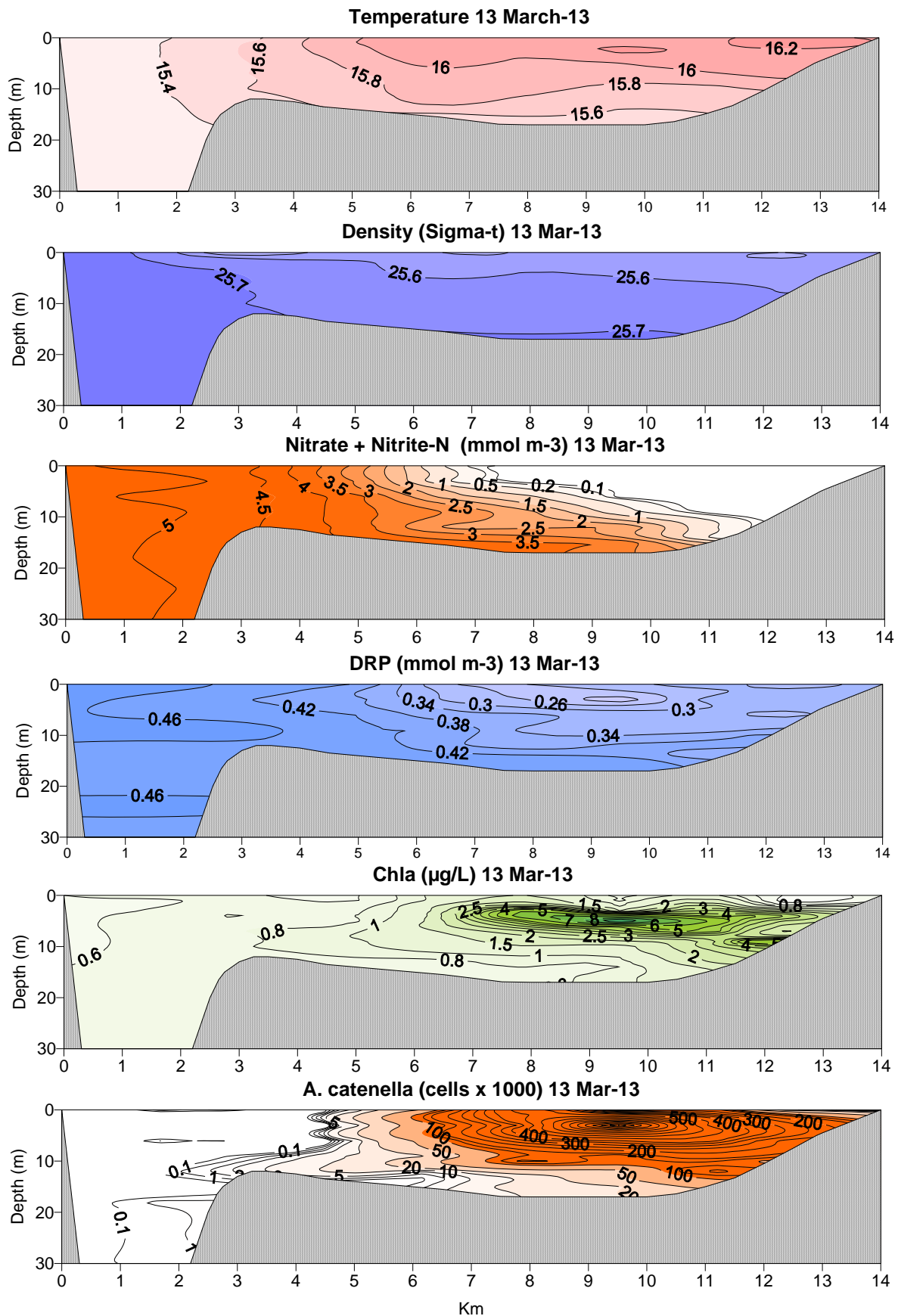


Figure 16. Tory Channel–Opua Bay transect sampled 13 March 2013.

4. QUEEN CHARLOTTE SOUND SHELLFISH CONTAMINATION

There was a close relationship between the appearance of *A. catenella* at Hitaua Bay, Tio Point and East Bay and the detection of PSP-toxins in Greenshell mussels at these sites (Figure 17). The timing of shellfish contamination at these locations was similar to that observed in 2011 (Mackenzie *et al.* 2011) though the magnitude and duration was much less in 2013. Because of this the duration of the harvest closure was less. In 2011 this began on 16 March and extended until 20 June at Hitaua Bay (97 days), and 1 April–20 June at East Bay (81 days). In 2013 Hitaua Bay and East Bay were closed on 25 March and reopened on 16 May (71 days).

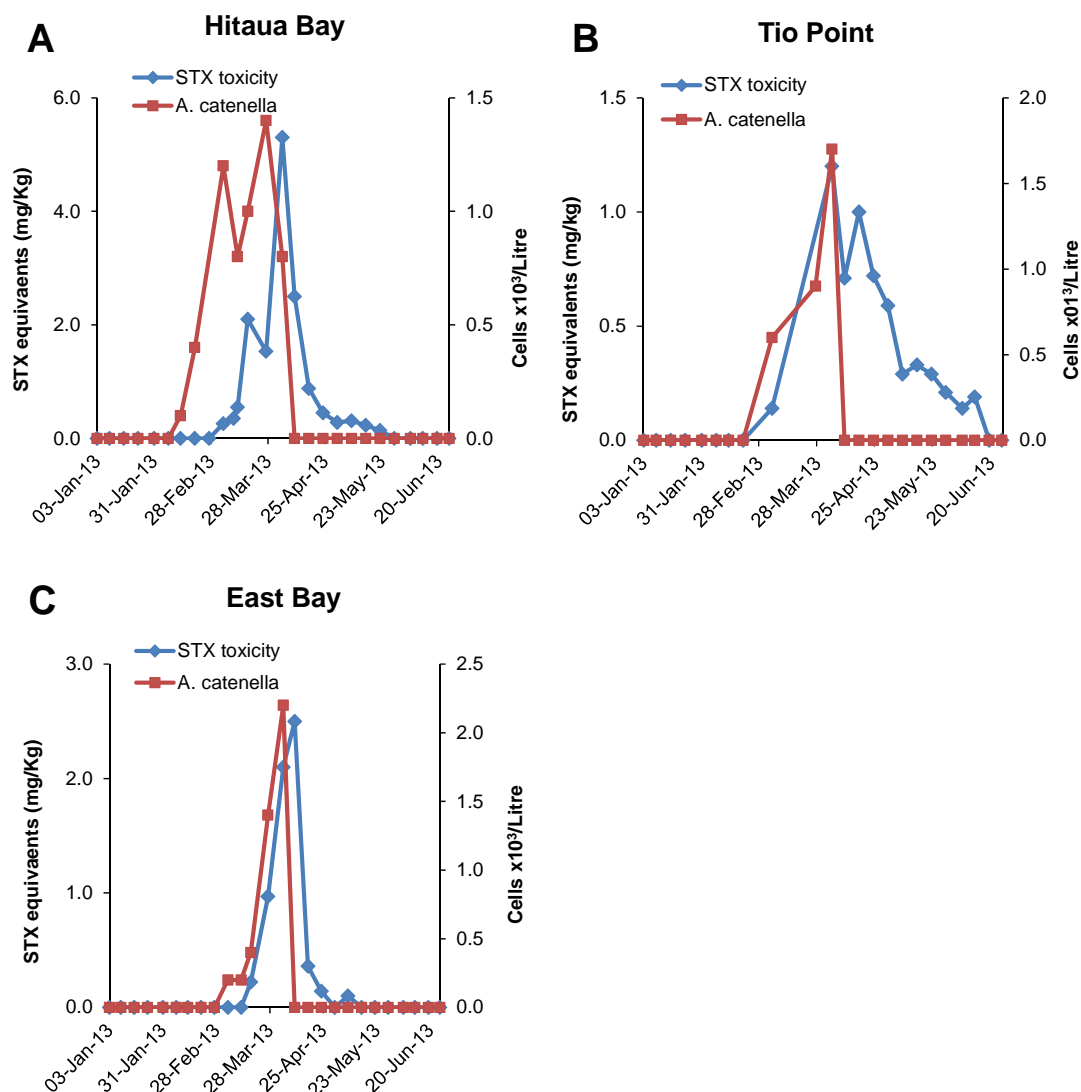


Figure 17. Paralytic shellfish poisoning-toxicity (PSP-toxicity) of Greenshell™ mussels (mg/kg STX equivalents) from the HPLC screen test, and *Alexandrium catenella* cell counts at Marlborough Shellfish Quality Programme (MSQP) monitoring sites in Queen Charlotte Sound, January–June 2013.



TOXIC SHELLFISH AREAS



DO NOT CONSUME SHELLFISH FROM THE AREA OUTLINED ON THIS MAP ()

This Area Is Affected By Shellfish Biotoxins.

Shellfish Consumed From This Area May Be Harmful To Health.

Scallops, Crayfish, Paua And Finfish May Be Gathered And Consumed If The Gut
(And Skirt Of Scallops) Is Removed.

For Further Information Contact:

NELSON MARLBOROUGH PUBLIC HEALTH SERVICE,
PH (03) 520 9914 BLENHEIM (03) 546 1537 NELSON (WORK HOURS)

OR

AFTER HOURS – THE ON-CALL HEALTH PROTECTION OFFICER,
(03) 520 9999 BLENHEIM OR (03) 546 1800 NELSON

DATE OF NOTICE: 25 March 2013 Queen Charlotte Sound & Tory Channel Closure
DATE OF NOTICE: 29 March 2013 Port Underwood Closure

Figure 18. Shellfish harvest closure notice for Queen Charlotte Sound (QCS) and Port Underwood, March 2013. Note. The Port Underwood closure was co-incidental with the QCS paralytic shellfish poisoning-toxin (PSP-toxin) event. It was due to diarrhetic toxin contamination of shellfish due to a bloom of *Dinophysis acuminata*.

Under the public health programme shellfish samples were also analysed for PSP-toxins from inner Queen Charlotte Sound sites (Onahau Bay, Whatamango Bay, Wedge Point and Momorangi Bay) in April and May. None of these samples returned positive results. Maximum screen PSP toxicity levels (STX equivalents) observed at Hitaua Bay, Tio Point and East Bay were 5.3, 1.2 and 2.5 mg/kg respectively. Bearing in mind that the confirmation / screen ratio for Greenshell mussels is around 0.35 (MacKenzie *et al.* 2012, Haywood *et al.* 2013), this translates into confirmed toxicity values of 1.8, 0.4 and 0.9 mg/kg, only a small margin above the regulatory level of 0.8 mg/kg. During the 2011 event maximum levels > 40 mg/kg (East Bay screen test) were recorded.

5. *ALEXANDRIUM CATENELLA* GROWTH RATE

To be able to predict the progress of the bloom, an accurate estimate of the population growth rate is important. An attempt was made to obtain a net *in situ* growth rate by sampling multiple sites with the bloom in Opuia Bay at various stages of its development. On four occasions counts were made of cells in 12 m integrated water column collected from 10 sites (Sites 5–14; Figure 19).

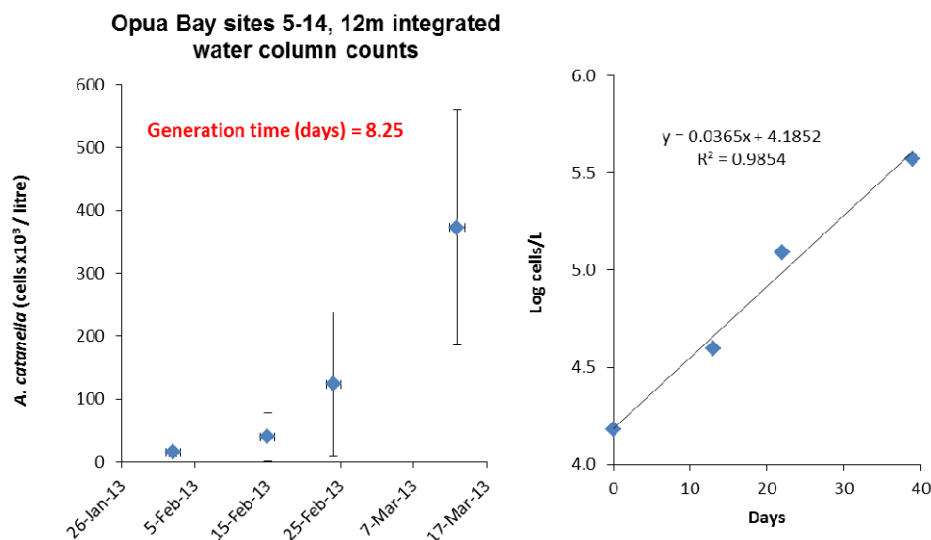
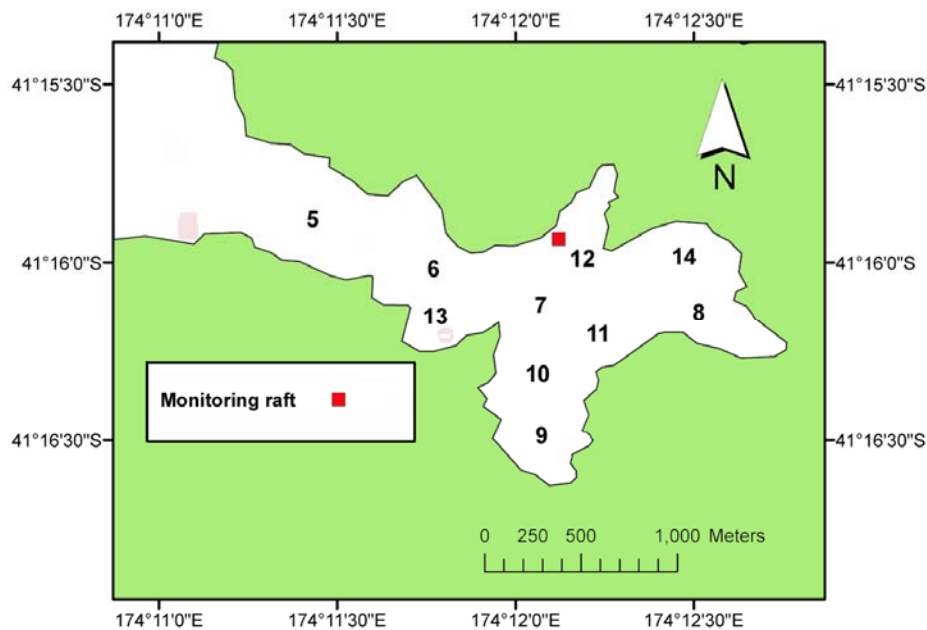


Figure 19. *In situ* net growth rate of *Alexandrium catenella* estimated from 12 metre integrated water column samples in Opuia Bay.

As the bloom reached a climax (Figure 19) it became increasingly patchy, both horizontally and vertically in the water column, and the variation around the mean abundance increased. From the growth curve a net population doubling time of 8.3 days was estimated. This is much lower than the maximum growth rate observed for *A. catenella* under optimum nutrient replete conditions in culture of around 1.5 days. It is also lower than a population doubling time of 5.2 days estimated the previous year (MacKenzie *et al.* 2012) from the weekly monitoring counts at Site 7, Opua Bay. Improving the accuracy of *in situ* net growth rate estimates will be a focus of future research.

6. *ALEXANDRIUM CATENELLA* IN NEW ZEALAND

Our knowledge of the New Zealand wide distribution of *A. catenella* has increased over the last two years (Figure 20) and is similar to that reported in 2011 (MacKenzie et al., 2011). *A. catenella* is most commonly observed on the east coast of the North Island from the Bay of Plenty northward and records of its occurrence in the Marlborough Sounds has increased.

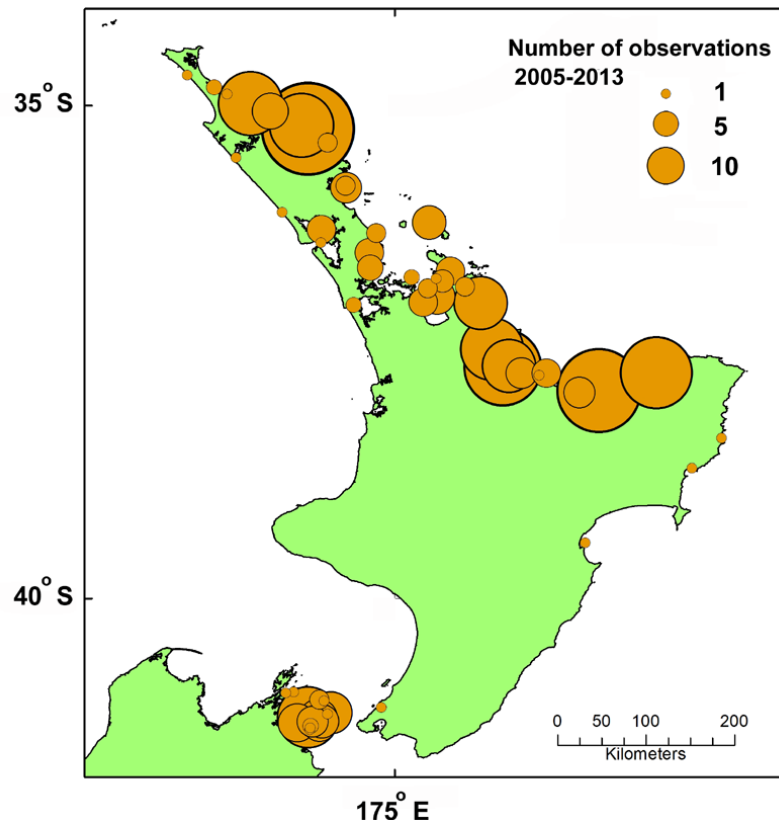


Figure 20. Locations and number of occasions *Alexandrium catenella* was observed in water samples collected weekly from around New Zealand, January 2005–June 2013. The Opuia Bay counts are excluded from the Marlborough Sounds data.

Over the last two years specimens have been detected for the first time in in the Manukau Harbour and a more frequently in the Kaipara Harbour. A minor bloom occurred during the spring of 2012 (Sept–Nov) in the outer Firth of Thames (Tamaki Strait, Waimangu Pt., Wilson Bay) in the vicinity of the Coromandel mussel farming area. The seasonal occurrence of *A. catenella* is more variable on the North Island east coast than in the Marlborough Sounds where main bloom period is between late February and the end of March. In January 2012 cell numbers up to 18,000 cells / L occurred in the Bay of Plenty (Tauranga, Bowentown and Tairua) but it did not re-

occur the following year. In Northland (Mangonui Wharf) moderate numbers of cells (1,900 cells/L) have been observed in July.

7. *MUNIDA GREGARIA* — A POTENTIAL VECTOR FOR FOOD CHAIN TRANSMISSION OF PSP-TOXINS

Munida gregaria (lobster krill, squat lobster) is a common seasonal inhabitant of cool temperate waters on New Zealand and South America. The Maori name for Tory Channel, Te Kura Te Au, translates as “the channel red with the blood of the octopus” (Te Ara Encyclopedia of New Zealand) and refers to swarms of *M. gregaria*, which in some years are abundant in the area. During the *A. catenella* bloom period (February–March, 2013) *M. gregaria* was very numerous throughout Tory Channel particularly within the Onepua/Opua Inlet (Fig. 21).

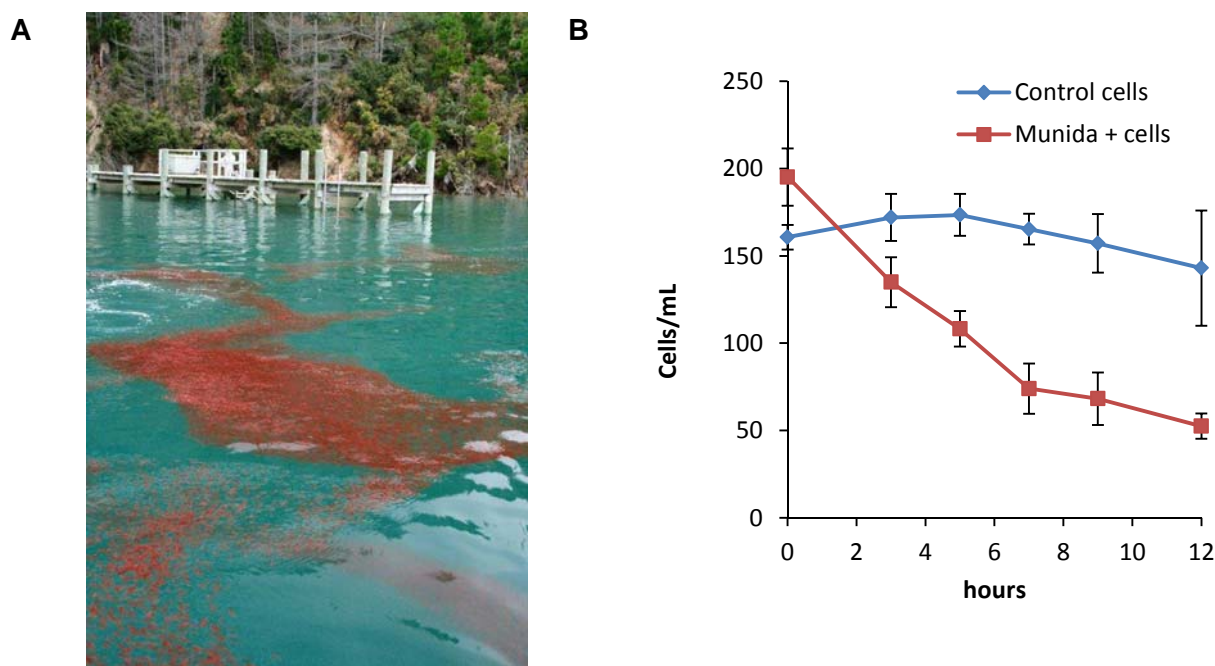


Figure 21. A) *Munida gregaria* swarm in Opua Bay).B) Experiment involving feeding *Munida gregaria* with *Alexandrium catenella* cells.

Analysis of extracts of *M. gregaria* specimens showed they contained PSP- toxins with an analogue profile similar to that of *A. catenella* (Figure 22) dominated by low toxicity N-sulfo carbamoyl analogues; C1,2 and GTX5 (B1) and GTX1,4.

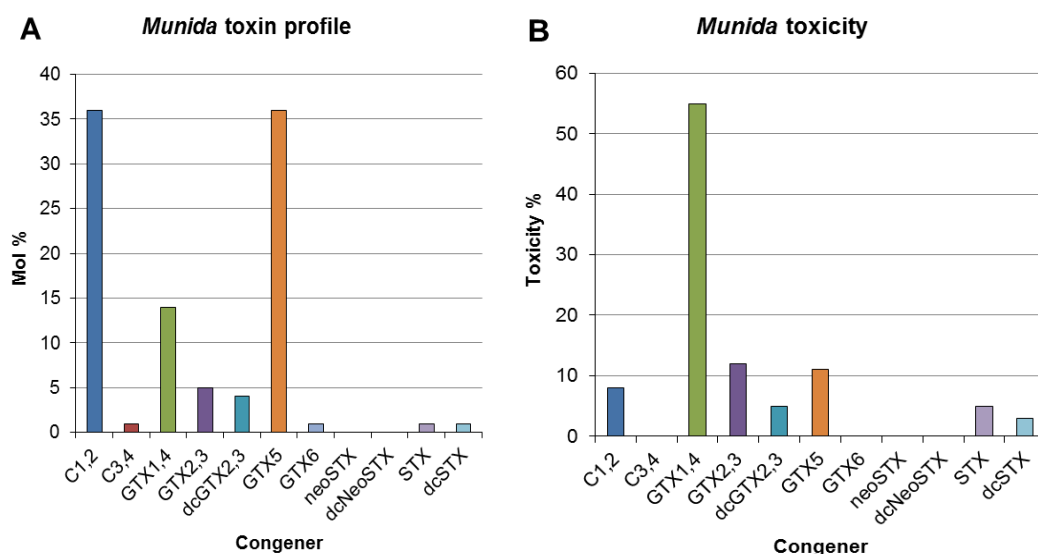


Figure 22. Analogue profiles and specific toxicity of paralytic shellfish poisoning-toxins (PSP-toxins) in *Munida gregaria* from Opuia Bay.

A feeding experiment was carried out to confirm that *M. gregaria* was actually capable of filtering *A. catenella* cells from the water (Figure 21 B). Seawater samples (triplicate 2 L samples) from Opuia Bay containing high numbers of *A. catenella* were placed in beakers with three *M. gregaria* individuals/beaker. The *A. catenella* cell numbers in these and control beakers were then monitored at 2-hourly intervals up to 12 hours. There was a significant decrease in the *A. catenella* cell numbers in the beakers containing *M. gregaria* over those in the control beakers (Figure 21 B). From this we conclude that *M. gregaria* is capable of directly grazing on *A. catenella* and is not necessarily accumulating the toxins by predation on other grazers such as copepods. To our knowledge this is the first time the accumulation PSP-toxins by *M. gregaria* has been observed.

It is conceivable that *M. gregaria*, may play a role in controlling *A. catenella* population growth. During sampling in Opuia Bay we observed that there were low numbers of *A. catenella* in the areas that *M. gregaria* were most concentrated and it is a possible reason why the calculated *in situ* population growth rate (Figure 19) was relatively low. When *M. gregaria* is abundant in the plankton it is an important food item for many animals, including fish, birds and whales and it is a hitherto unrecognised means by which PSP-toxins can be transmitted through the food chain. *M. gregaria* individuals were found in the stomachs of blue cod caught in the area at the time of the bloom.

8. *ALEXANDRIUM CATENELLA* CYST ANALYSIS OF OPUA BAY SEDIMENT CORES

In a previous report (MacKenzie *et al.* 2012), we presented the results of *A. catenella* resting cyst counts within a sediment core, subsampled from a van Veen grab sample from Opuia Bay. In this core we found variable cysts down to at least 10 cm (the maximum depth of the core), suggesting that the dinoflagellate was not a recent arrival in the Bay. However because of the limited depth of the core and the way in which it was obtained we felt we needed to repeat these analyses on deeper cores collected in a more rigorous fashion by SCUBA divers. Several 40 cm deep cores sampled in this way were collected in December 2012 and cysts counts made on 1 cm sediment slices using the primuline staining method (Yamaguchi *et al.* 1995). Care was taken to prevent contamination by thoroughly cleaning equipment between cutting each section and subsampling from the centre of each slice.

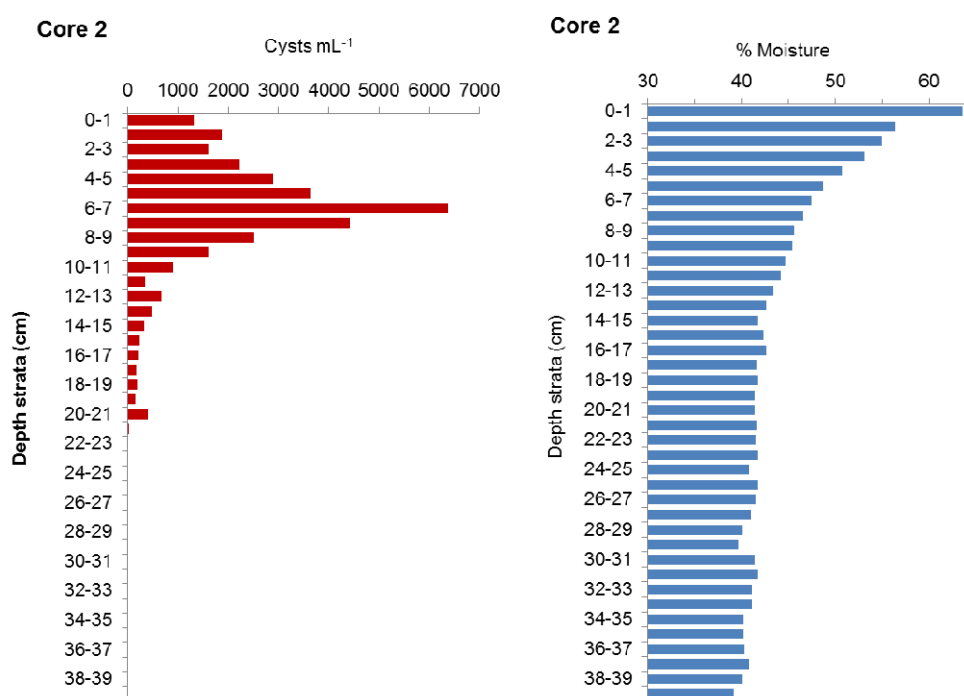


Figure 23. The vertical distribution *Alexandrium catenella* resting cysts in a core collected by SCUBA from Site 7, Opuia Bay, December 2012.

Two cores were analysed and in each one *A. catenella* cysts were found down to a depth of at least 20 cm (Figure 23). We are still waiting for the results of isotope (²¹⁰Pb and ¹³⁷Cs) analyses being carried out by the Institute of Geological and Nuclear Sciences (GNS) which we hope will provide an estimate of the age of the various depth strata within the core. Nevertheless it is reasonable to suppose that a depth of 20cm is well below the reach of recent bioturbation and that we can conclude that

A. catenella has been blooming seasonally and depositing cysts in Opau Bay for at least several decades, if not longer. *Alexandrium* spp. cysts at 100cm depth in cores from Sequin Bay Puget Sound, USA have been dated to the late 1800s (Feifel *et al.* 2012).

9. BAY OF PLENTY SHELLFISH POISONING EVENT, DECEMBER 2012

During the week of 10 December 2012, at least 29 people became ill from consuming shellfish (mainly Tuatua) collected from BOP beaches between Mt Maunganui and Papamoa. Seventeen people were admitted to hospital and at least two serious cases required treatment in the intensive care unit for several days (ESR 2012). High numbers of *Alexandrium minutum* were observed in the phytoplankton (Figure 24), the patients exhibited classic symptoms of paralytic shellfish poisoning (PSP), specimens from a batch that caused human poisoning contained high levels of STX and neoSTX (Figure 25) and toxin residues were identified in the urine from an affected individual (Cawthron unpublished data). This was the most serious PSP event that has been documented in New Zealand to date, although there is evidence of previous poisonings in the Bay of Plenty (ESR 2010)

A public health toxic-shellfish warning was in place in the area when the poisoning took place. *Alexandrium minutum* had appeared in the plankton (200–300 cells/L) at the Bowentown Wharf and Tauranga Harbour monitoring sites (Figure 24) on 11 November increasing to 1,100-5,900 cells/L by 18 November. Concentrations of PSP-toxins (1.6 mg/kg) over the regulatory level of 0.8 mg/kg were first observed in Tuatua on Pukehina and Papamoa beaches on 25 November and 2 December respectively (Figure 24; AssureQual data). The Bay of Plenty has a history of shellfish contamination by toxins from *Alexandrium* species and the December 2012 event was very similar to an event attributable to *A. minutum* in January 1993 (MacKenzie *et al.* 1997). Testing of Tuatua that were involved in the Bay of Plenty poisoning incident showed that these contained high levels of PSP toxins. Cawthron analysis of a sample collected from Papamoa Beach (near the Omanu Surf Club) on 16 December 2012 had concentrations of 31 mg STX equiv'/kg and 14 mg STX equiv'/kg on the Lawrence HPLC screen and confirmation tests respectively. The confirmation level was > 17 times the regulatory limit and the toxin profile was dominated by GTX1,4, STX and neoSTX, (Figure 25 A). These toxicity estimates were considerably higher than the values reported from the routine monitoring samples from Papamoa and Pukehina beaches (~4.0 mg/kg STX equivalents; Figure 24) and calls into question the accuracy of the latter analyses.

The high levels of GTX1,4, STX and neoSTX in the Bay of Plenty Tuatua (Figure 25), originating from *Alexandrium minutum* (Figure 26), were undoubtedly the reason for

the human poisoning incident in January 2013. Tuatua are the main bivalve species gathered along the extensive sandy surf beaches in the Bay of Plenty and they have the propensity to sequester PSP-toxins (predominately in their syphons) for long periods after the contamination phase is over (MacKenzie *et al.* 1996). Tuatua also may convert less toxin analogues (e.g. GTX1,4) to more toxic forms (STX, neoSTX)

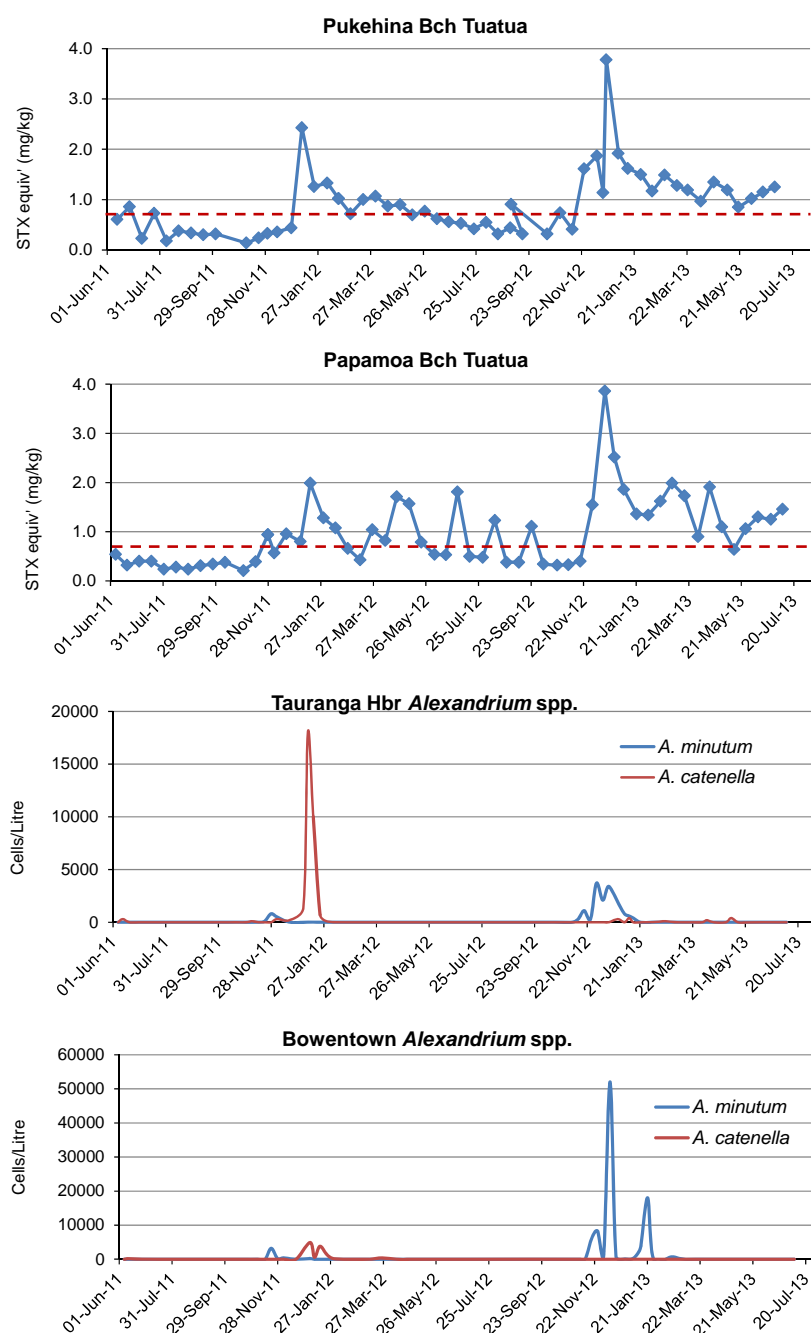


Figure 24. Paralytic shellfish poisoning-toxicity (PSP-toxicity) scores (AssureQuality HPLC analyses) in Tuatua (*Paphies subtriangulata*) and cell abundances of *Alexandrium catenella* and *Alexandrium minutum* at Bay of Plenty monitoring sites, June 2011–June 2013.

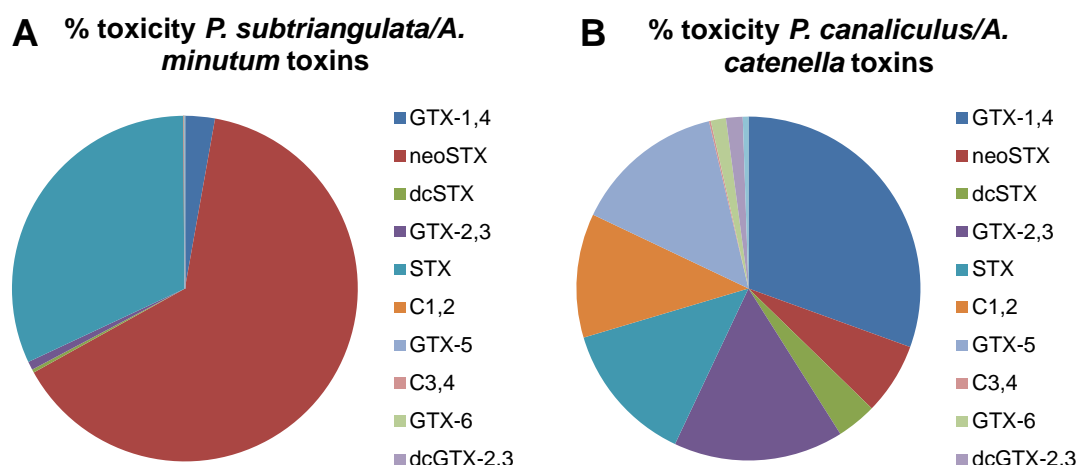


Figure 25. Toxin profiles (% contribution to total toxicity) of Tuatua (*Paphies subtriangulata*) contaminated with paralytic shellfish poisoning-toxicity (PSP-toxins) from *Alexandrium minutum* (A) and GreenshellTM mussels (*Perna canaliculus*) contaminated with *Alexandrium catenella* toxins (B). The Tuatua were collected from Papamoa Beach in December 2012, the GreenshellTM mussels were from Queen Charlotte Sound, May 2011.

Alexandrium catenella is a common member of the phytoplankton in the Bay of Plenty and is probably the main cause of historical PSP contamination events (e.g. Figures 24 and 27-28). It has been responsible for high toxicity levels in mussels and clams on a number of occasions, however; the incidence of human poisoning resulting from these events appears to be low. Analysis of cultured isolates of *A. catenella* from Queen Charlotte Sound and the Bay of Plenty (MacKenzie *et al.* 2004) have shown that these have similar toxin profiles with a high proportion of low specific toxicity N-sulfo-carbonyl congeners predominately C1 and C2 and GTX5. It is believed that this is the reason why there is an apparently low rate of morbidity associated with *A. catenella* blooms in the Bay.

In summary; the outbreak of PSP-poisoning in the Bay of Plenty in December 2012 was due to a bloom of *Alexandrium minutum* which contaminated shellfish with high levels of the most toxic PSP-toxin analogues STX and neoSTX (see Appendices 1 and 2). Although a public health warning was in place, this was clearly ineffective in preventing people from harvesting dangerously contaminated shellfish. A contributing factor may be that the public has come to ignore warnings because most PSP contamination events in the Bay are due to *Alexandrium catenella* blooms which predominately contaminate shellfish with lower toxicity analogues. Unless present in very high concentrations these are less likely to cause illness.

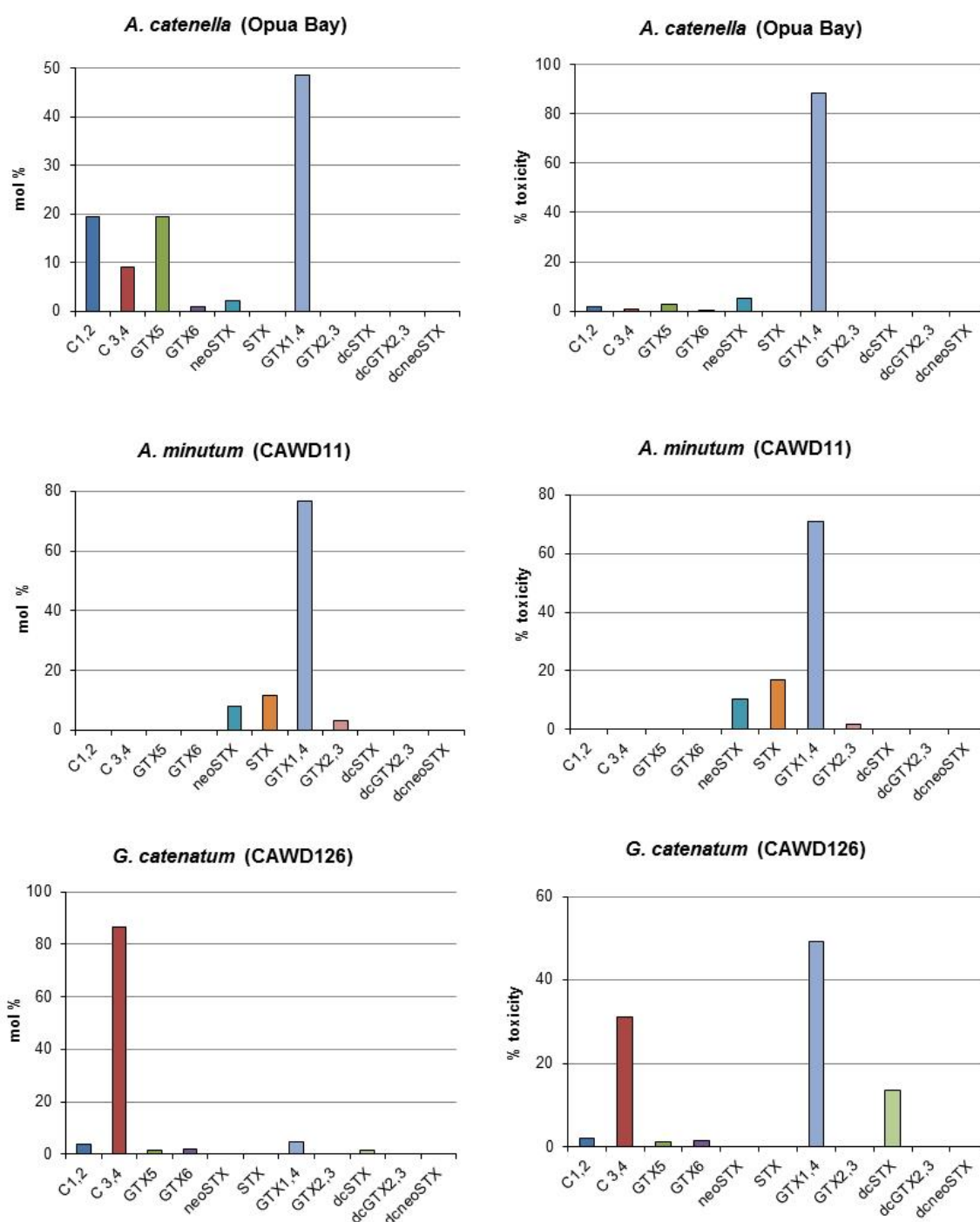


Figure 26. PSP-toxin profiles in cultured isolates of *Alexandrium catenella* (Opua Bay isolate), *Alexandrium minutum* (Anakoha Bay, Marlborough Sounds isolate; culture collection # CAWD11) and *Gymnodinium catenatum* (Manukau Harbour. isolate; culture collection # CAWD126).

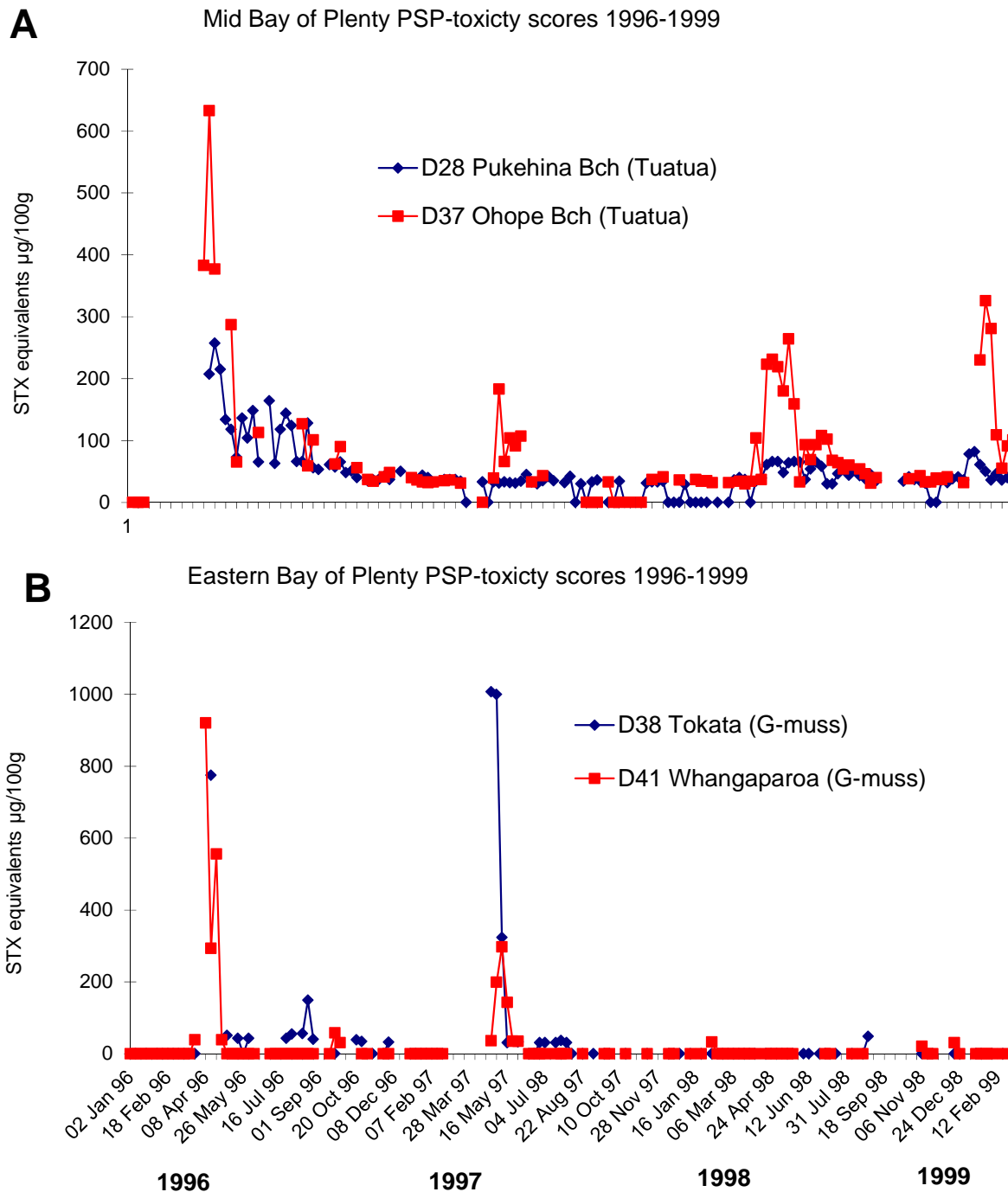


Figure 27. Paralytic shellfish poisoning-toxicity (PSP-toxicity) scores at monitoring sites in the mid, A) and eastern, B) regions of the Bay of Plenty 1996–1998, associated with blooms of *Alexandrium catenella*. The toxicity was determined by mouse bioassay and the results are expressed as μg STX equivalents /100g of shellfish flesh. The Tokata (D38) and Whangaparaoa (D41) sites are no longer routinely sampled as part of the public health biotoxin surveillance programme.

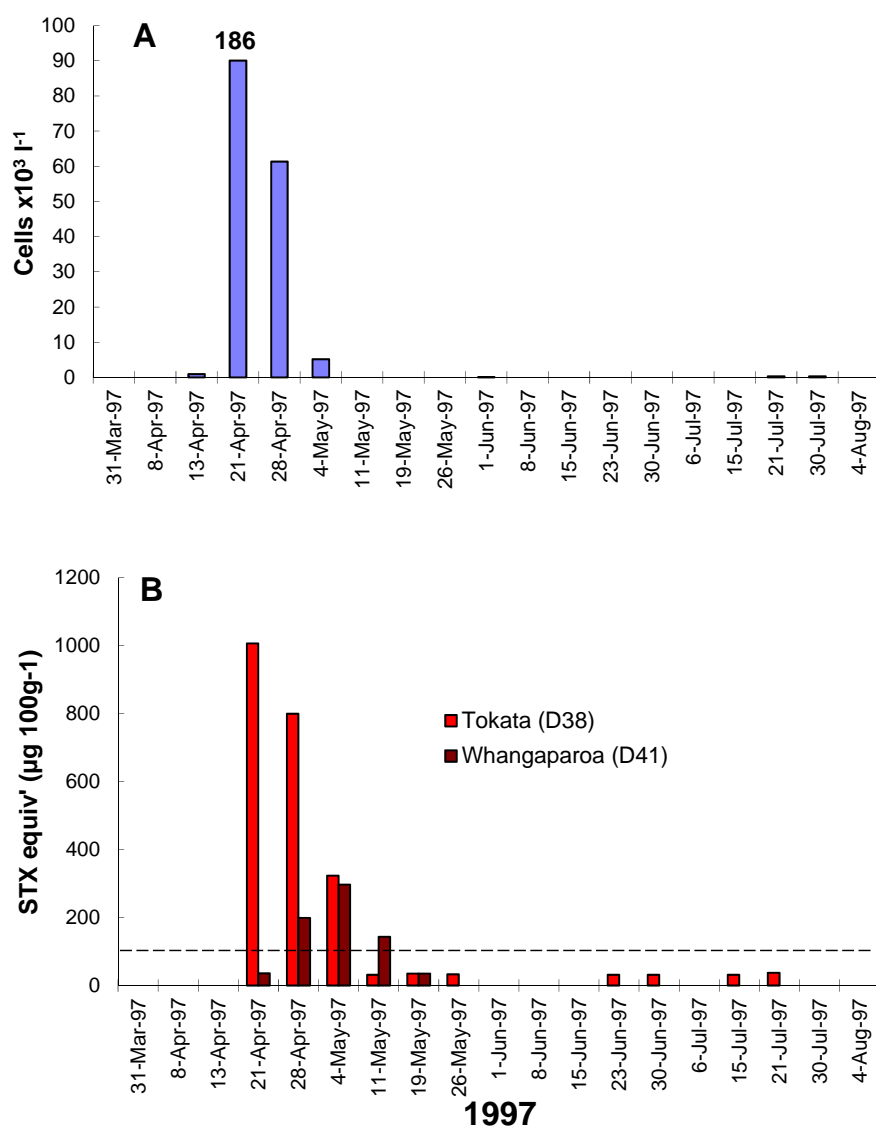


Figure 28. *Alexandrium catenella* and paralytic shellfish poisoning-toxicity (PSP-toxin) contamination of wild Greenshell™ mussels in the eastern Bay of Plenty (Whangaparoa and Tokata), March–July 1997. A) Cell numbers of *A. catenella* in the water column at Tokata. B) Saxitoxin equivalents ($\mu\text{g}/100\text{g}$) determined by mouse bioassay.

10. *GYMNODINIUM CATENATUM* BLOOM ON THE NORTH ISLAND WEST COAST, AUGUST-DECEMBER 2012

In late winter and spring 2012 the toxic dinoflagellate *Gymnodinium catenatum* appeared in water samples and low levels of PSP-toxins were detected in shellfish, at a number of public health monitoring sites on the North Island west coast. This species had not been observed in this region since a few cells were last reported in 2007.

Between May 2000 and February 2001 a bloom of the toxic dinoflagellate *Gymnodinium catenatum*, a species previously not known in New Zealand waters, led to the widespread contamination of shellfish with PSP toxins around the coast of the North Island. This event was the most extensive, and one of the most harmful, toxic algae blooms to be documented in New Zealand at that time (MacKenzie & Beauchamp, 2001). There were few if any accounts of human illness associated with the event, however it led to health authorities issuing warnings to the public to refrain from collecting shellfish from over 1,500 km of coastline, for periods of up to nine months in some areas.

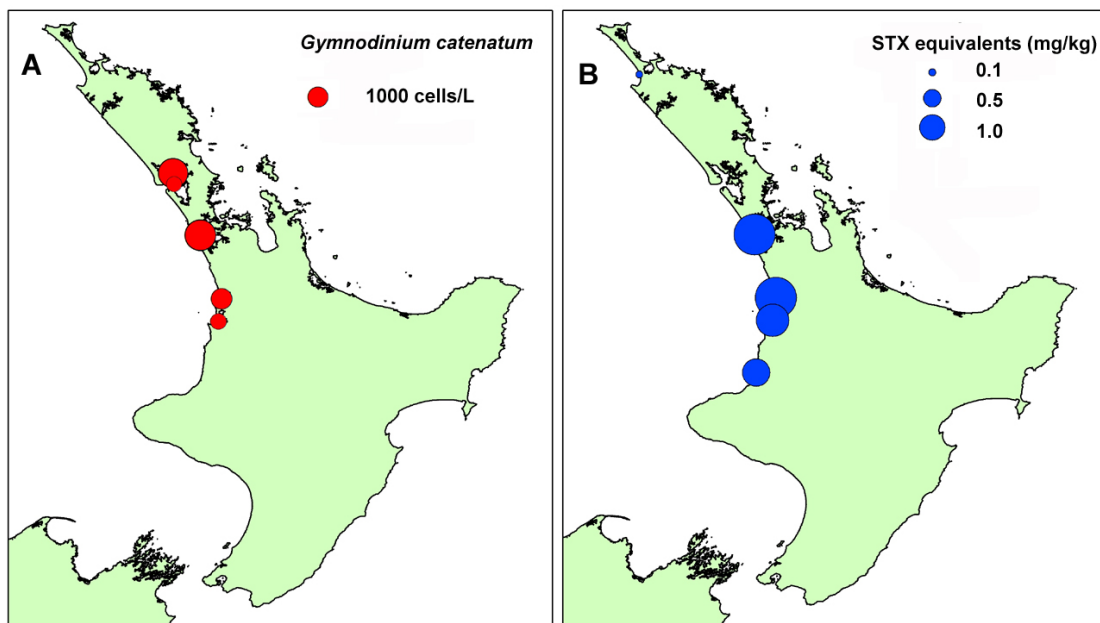


Figure 29. The distribution of *Gymnodinium catenatum* cells, A) and associated PSP-toxicity, B) on the North Island west coast, August–December 2012. The symbols are proportional to the maximum values observed at these sites over this period.

Although the main commercial shellfish growing areas 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, primarily due to the risk of trans-locating *G. catenatum* resting cysts. Unfortunately much of the premium seed source for the oyster and mussel industries lay within affected west coast areas and as a result production of the Marlborough mussel industry was seriously affected.

The 2000 bloom developed offshore on the north western coast of the North Island in the vicinity of, the Manukau Harbour in May and spread north and south from there. The bloom eventually extended down the North Island west coast, through Cook Strait and up the east coast of the North Island at least as far as Hawke's Bay.

Gymnodinium catenatum persisted but slowly dwindled in abundance in the phytoplankton on the North Island west coast and in Hawkes Bay for several years after the initial bloom in 2000. Low but detectable levels of PSP-toxin (measured by mouse bioassay) occurred in shellfish from Gisborne and the Kaipara Harbour up until at least May 2004. Until August 2012, the last records of *G. catenatum* in the phytoplankton data base were from Hawke's Bay and the Kaipara Harbour in 2007.

Table 2. PSP-toxins in shellfish from North Island west coast monitoring sites probably attributable to *Gymnodinium catenatum* (AssureQual data).

Week ended	Waipapakauri	Huia Bank Manukau Hbr	Raglan Hbr	Kawhia Hbr	Mohakatino
STX equivalents mg/Kg					
17 Aug-12			0.41	0.65	
7 Sept-12		0.29			0.19
14 Sept-12			0.23	0.13	
21 Sept-12		0.11			0.31
28 Sept-12		0.14	0.38	0.59	
5 Oct-12		0.24			0.42
12 Oct-12		0.45	0.21	0.14	
19 Oct-12	0.1	0.44			0.47
26 Oct-12				0.83	
2 Nov-12		0.22	1.1		

Table 3. Numbers of *Gymnodinium catenatum* cells in water samples from the North Island west coast monitoring sites September–November 2012.

Week ended	Kaipara Tinopia	Kaipara Tabora	Huia Bank Manukau Hbr.	Raglan Hbr.	Kawhia Hbr.
<i>G. catenatum</i> cells/L					
21 Sept-12			500		
5 Oct-12			100		
19 Oct-12			2100		
26 Oct-12				1100	700
2 Nov-12			900		
16 Nov-12			1700		
23 Nov-12	1900				
30 Nov-12		600	1500		

In 2012 only limited water and shellfish sampling was carried out on the west coast (e.g. fortnightly at Huia Bank, Manukau Harbour) over the bloom period (Tables 1 and 2). The data from this sampling (Table 2) provides some idea of its intensity and progression. Cells of *G. catenatum* (500 cells/L) were first observed in a seawater sample from Huia Bank, Manukau Harbour on 16 September 2012, though sometime prior to this (week of 17 August 2012), low levels of PSP-toxicity (0.4-0.7 mg STX equiv' /kg) had been identified in shellfish from Raglan and Kawhia harbours. *G. catenatum* persisted in the plankton in the Manukau Harbour until 24 November 2012 reaching a maximum of 2100 cells/L on 15 October 2012. It was not seen (700-1100 cells/L) in samples from Kawhia and Raglan harbours until 24 October 2012.

In contrast, in 2001 cell numbers as high as 276,000 cells/L and toxicity levels of > 40 mg STX equivalents/kg (mouse bioassay) were observed at some west coast sites.

It is notable that the appearance of this dinoflagellate occurred in the same locations and at approximately the same time of year (*i.e.* winter) after an apparent complete absence of around six years. In this case the bloom did not develop and spread to anything like the extent it did in 2001, but it suggests that a similar event could occur again, originating from the same region.

11. ACKNOWLEDGEMENTS

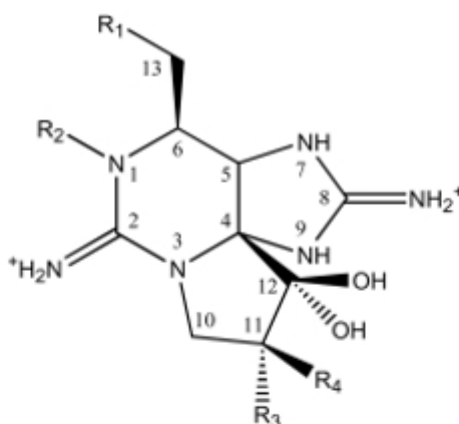
Thanks to the Marlborough Shellfish Quality programme (Helen Smale, Noel McArthur, Mike Williams) for carrying out the weekly sampling at Opuia Bay, and for the use of the Queen Charlotte Sound shellfish toxicity and phytoplankton monitoring data. Thanks also to NZ King Salmon Company Ltd. for access to their phytoplankton monitoring data. Routine analysis of Opuia Bay phytoplankton samples and field work and analyses involved in the Tory-Opuia Bay transects were funded through Cawthron Institute's Seafood Safety Research Programme (MBIE contract CAX0703). Allie Tonks who carried out all the *A. catenella* cyst analyses was funded through a Bayer Boost Scholarship.

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13. APPENDICES

Appendix 1. Molecular structure of paralytic shellfish poisoning-toxicity (PSP-toxin) analogues.



R-1	R-2	R-3	R-4	Compound Name
Carbamates	-H	-H	-H	STX
$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}-\text{NH}_2 \end{array}$	-OH	-H	-H	neoSTX
	-OH	$-\text{OSO}_3^-$	-H	GTX-1
	-H	$-\text{OSO}_3^-$	-H	GTX-2
	-H	-H	$-\text{OSO}_3^-$	GTX-3
	-OH	-H	$-\text{OSO}_3^-$	GTX-4
N-sulfocarbamates	-H	-H	-H	GTX-5
$\begin{array}{c} \text{O} \quad \text{H} \\ \parallel \quad \\ -\text{C}-\text{O}-\text{N}-\text{SO}_3^- \end{array}$	-OH	-H	-H	GTX-6
	-OH	$-\text{OSO}_3^-$	-H	C-3
	-H	$-\text{OSO}_3^-$	-H	C-1
	-H	-H	$-\text{OSO}_3^-$	C-2
	-OH	-H	$-\text{OSO}_3^-$	C-4
Decarbamoyl toxins	-H	-H	-H	dcSTX
	-OH	-H	-H	dcneoSTX
	-OH	$-\text{OSO}_3^-$	-H	dcGTX-1
-OH	-H	$-\text{OSO}_3^-$	-H	dcGTX-2
	-H	-H	$-\text{OSO}_3^-$	dcGTX-3
	-OH	-H	$-\text{OSO}_3^-$	dcGTX-4
Deoxy toxins	-H	-H	-H	doSTX
	-OH	$-\text{OSO}_3^-$	-H	doGTX-2
-H	-OH	-H	$-\text{OSO}_3^-$	doGTX-3

Appendix 2. Relative toxicities of saxitoxin (STX) analogues produced by *Alexandrium* spp. (after Oshima, 1995).

Toxin analogue	Relative toxicity
C1	0.01
C2	0.10
C3	0.01
C4	0.06
GTX1	1.00
GTX2	0.36
GTX3	0.64
GTX4	0.73
GTX5 (B1)	0.06
GTX6 (B2)	0.06
Neo STX	0.92
STX	1.00