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An *Alexandrium catenella* bloom and associated saxitoxin contamination of shellfish, Queen Charlotte Sound, March-June 2011

RESEARCH BASED SOLUTIONS

An *Alexandrium catenella* bloom and associated saxitoxin contamination of shellfish, Queen Charlotte Sound, March-June 2011

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Prepared for

MAF Food Safety

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EXECUTIVE SUMMARY

- Beginning in early March 2011, a bloom of the saxitoxin (STX) producing dinoflagellate *Alexandrium catenella* developed in Tory Channel and spread throughout the greater part of the Queen Charlotte Sound.
- The dinoflagellate bloom itself persisted until mid –late April, although the prohibition of commercial shellfish harvesting did not end at East Bay until 20 June 2011. The maximum closure period was 97 days.
- Low numbers of the dinoflagellate had been observed in the same locations between February and May 2010, together with STX concentrations below 0.8 mg/kg STX equivalents in mussels.
- *Alexandrium catenella* is common on the north east coast of the North Island, especially in the Bay of Plenty and Bay of Island regions, where there is a history of recurrent blooms.
- Solitary *A. catenella* cells have been observed in outer Pelorus Sound and Port Underwood. It cannot be predicted whether major blooms will develop in these areas in the future, but this more likely now than in the past.
- A survey has found that *A. catenella* resting cysts are now widespread around Queen Charlotte Sound, though numbers are generally low. The highest cyst densities were found in the sediments of Opua Bay and it is likely these will provide the seed source for regeneration of the bloom next year. It is recommended that Opua Bay be made an additional phytoplankton monitoring site.
- As a precaution against spreading cysts it is important that sediments especially from the Tory Channel area are not transported (e.g. on anchors) to unaffected areas such as Port Underwood and Pelorus Sound.
- Fortunately the suite of STX analogues produced by the dinoflagellate are dominated by the low toxicity C1,2 types and this is likely to continue to be the case in future blooms. Nevertheless the levels of total STX measured in shellfish are amongst the highest ever recorded in New Zealand.

- The bloom was a good test of the newly established chemical analysis programme (based on the Lawrence method) for saxitoxins, officially sanctioned and introduced for routine monitoring in April 2010. Despite its complexity the analytical programme performed well. This is believed to be the first time this method has been used to manage a PSP-toxin event anywhere in the world.

- Flat oysters did not accumulate STXs (maximum 7.9 mg/kg STX equivalents) to levels as high as in Greenshell and Blue mussels (maxima 41.5 and 25 mg/kg STX equivalents respectively).

- The mean ratio of confirm/screen estimates of total STX toxicity was 0.35 ± 0.08 and 0.39 ±1 0.14 for *O. chilensis* (oyster) and *P. canaliculus* (Greenshell mussel) respectively. These values provide a good rule of thumb for estimating real toxicity from screen test results and deciding when to initiate confirmation testing.

- The toxin depuration data suggests that in the late depuration phase, as long as screen test results are below 0.8 mg/kg and are remaining constant or continuing a downward trend, there is a good degree of certainty that the real toxicity will be below 0.4 mg/kg STX equivalents, and additional confirmation tests should not be necessary.

- The efficacy of qPCR molecular probes for semi-automated enumeration of motile cells was evaluated and showed promising results.

- Water column CTD-fluor profiles in Tory Channel were measured at the height of the bloom and a variety of other environmental data was collected. Seawater temperatures in Tory Channel were around 0.5-1.0 degrees lower than the average during and preceding the bloom. The Easter weekend storm which ended the bloom coincided with a rapid drop in seawater temperature.

- If *A. catenella* becomes established in the main mussel growing regions it may develop into a chronic annual problem causing serious disruption the industry's harvesting operations.

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1. INTRODUCTION

Contamination of shellfish with paralytic shellfish poisoning (PSP) toxins (saxitoxins) produced by planktonic dinoflagellates (*Alexandrium* spp., *Gymnodinium catenatum* and few other species) is a world-wide public health and shellfish quality assurance problem. Saxitoxins (STXs) are regarded as amongst the most serious of the many marine biotoxin types because some of the compounds involved are potentially lethal. There have been few accounts of illness associated with saxitoxins in New Zealand, although six cases of suspected PSP-poisoning (3 cases requiring hospitalisation) from the consumption of clams (Pipi and Tuatua) in the Bay of Plenty were notified in January 2010 (ESR 2010).

Since routine monitoring began in the early 1990s it has become apparent that *Alexandrium* spp. are common in New Zealand coastal waters, but significant blooms are infrequent and saxitoxins in shellfish relatively rare. The east coast of the North Island, especially the Bay of Plenty and Bay of Islands are known hotspots for *Alexandrium* spp. blooms although the biggest saxitoxin event recorded in New Zealand was that caused by an extensive bloom of *Gymnodinum catenatum* in 2000-2001 which spread down the North Island west coast and up the east coast as far as the Bay of Plenty (MacKenzie and Beauchamp 2001). *G. catenatum* was abundant for a time in Cook Strait during this bloom and low numbers of cells were seen in the Marlborough Sounds but fortunately it did not become permanently established.

Saxitoxin contamination of shellfish in the Marlborough Sounds has occurred in the past due to minor blooms of *Alexandrium minutum,* including several incidents requiring the closure of mussel harvesting in Croiselles Harbour in 1993 and in Anakoha Bay, Pelorus Sound in January 1994 (MacKenzie 1994, MacKenzie and Berkett, 1997). On the latter occasion maximum shellfish toxicity (determined by mouse assay) and cell numbers reached 1.3 mg/kg STX equivalents and 1.1 $x10^5$ cells/litre respectively.

On 9th March 2011, cells of *Alexandrium catenella* were first seen in monitoring samples from Hitaua Bay, Tory Channel, concurrent with the detection of STXs at a concentration over the action level (>0.4 mg/kg) in mussels from this location. The next week cells were observed at Tio Point and toxins were detected in cultured oysters. Over the following 3-4 weeks the bloom increased in intensity and extent, spreading throughout Queen Charlotte Sound accompanied by high levels of STXs in a variety of shellfish. Extremely high numbers of cells (>4 million cells/litre) caused a red- orange discolouration of the water ("red tide") at some locations.

Until recently the testing for STXs in shellfish for regulatory purposes was almost exclusively based on the A.O.A.C. mouse bioassay (MBA). The MBA is a simple, low tech method but for variety of good technical and ethical reasons (Holland et al., 2010a) a shift to routine testing using a chemical analysis method (AOAC, 2005; Holland et al., 2010b) was authorized by the NZ Food Safety Authority in 2010 and came into routine use in April of that year. The Queen Charlotte Sound *A. catenella* event was the first time this method has been used in New Zealand to detect and determine the spatial extent of a saxitoxin bloom and manage the harvest closure and re-opening decisions. It has provided an excellent technical test of the method

itself and provided useful quantitative toxin data to evaluate opening and closure criteria and procedures.

This report collates the plankton and shellfish-toxin data gathered during the event. It provides a detailed description of the development and decline of the bloom and associated shellfish contamination and depuration phases. It discusses the possibility that the dinoflagellate may spread throughout the Marlborough Sounds and become a permanent component of the regional phytoplankton.

1.1. *Alexandrium catenella* in New Zealand

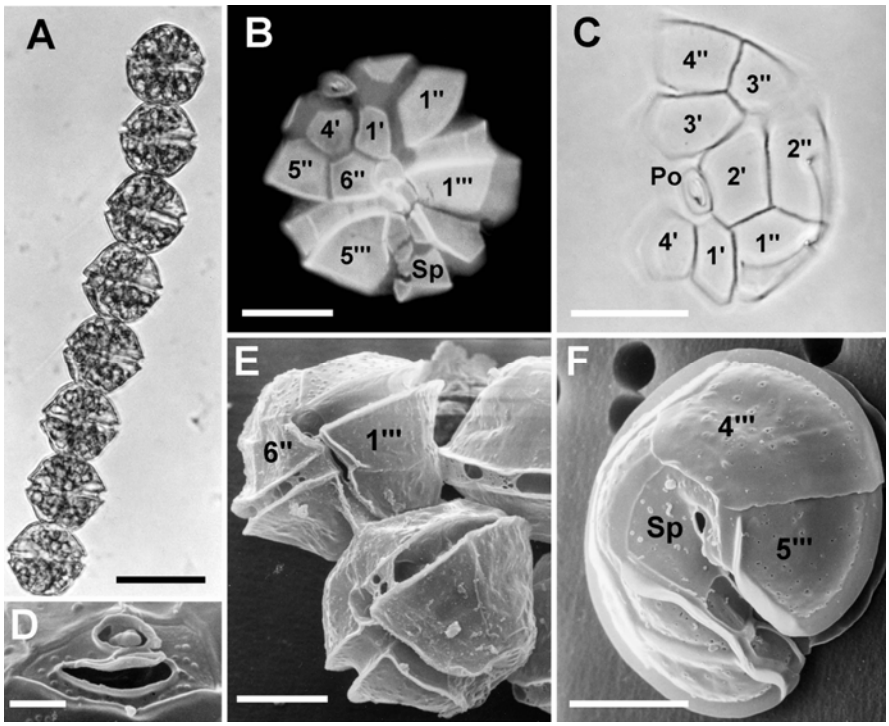


Figure 1. Morphology of *Alexandrium catenella* (from MacKenzie et al. 2004)

Alexandrium catenella (Figure 1) has often been observed in water samples from the north east coast of the North Island and blooms are not uncommon throughout this region (Figure 2). *A. catenella* was first seen in samples from Waihi Beach and Tauranga Harbour in the Bay of Plenty on 28 April 1996 (MacKenzie et al. 2004) in association with elevated levels of PSP-toxins (detected by MBA) in mussels and surf clams in the middle and eastern sections of the bay. In April 1997 cell numbers up to 1.9 x10^5 cells/litre were detected in surface waters at Te Kaha (eastern Bay of Plenty) and the toxicity in Greenshell mussels affected by this bloom reached a maximum of 10.1 mg STX equivalents/kg. In September-October 2007 another bloom occurred in the same area extending from at least Te Kaha in the east to Tairua in the western bay with cell numbers up to 2.3 x10^5 cells. Cell numbers of *A. catenella* over the trigger levels have been observed at Tirua in the Bay of Plenty as recently as 19 June 2011.

HPLC analysis of fourteen *A. catenella* cultures of cells isolated from the Bay of Plenty showed a high degree of uniformity between them in terms of their toxin profiles (MacKenzie et al. 2004). These were dominated by almost equal proportions of the low toxicity *N*-sulfo-carbamoyl analogues (C_1 + C_2 and C_3 + C_4) with minor amounts of GTX4, GTX1 and neoSTX.

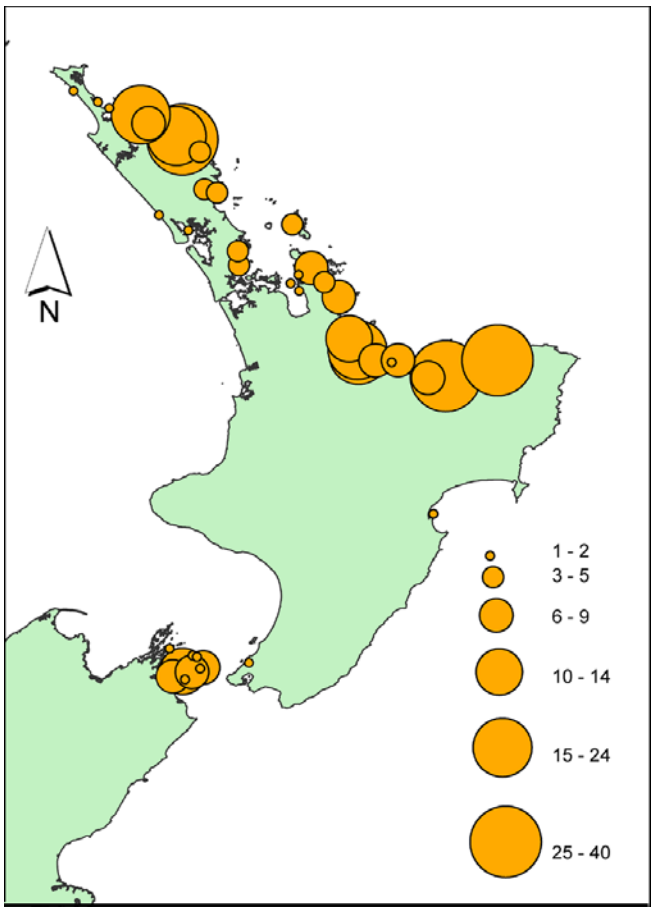


Figure 2 Locations and the number of occasions *Alexandrium catenella* was observed in water samples collected weekly from around New Zealand, January 2005 – June 2011. The data from the Marlborough Sounds were mainly collected during the 2011 bloom.

Occasionally low numbers of *A. catenella* have been observed in the Hauraki Gulf and Firth of Thames, including the Coromandel mussel farming area (Wilsons Bay, Motukopake). The dinoflagellate has been observed in the Bay of Islands on numerous occasions (Figure 2) and in April 2003, January 2007 and December 2007 blooms of *A. catenella* (> 1.0 x10^4 cells/litre) occurred at Tapeka Point associated with PSP-toxin contamination of cultivated oysters. There are a few records of its occurrence in Hawkes Bay and at several locations on the North Island west coast.

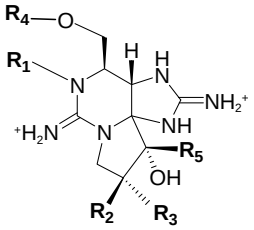
1.2. *Alexandrium catenella* and PSP-toxins in the Marlborough Sounds

Alexandrium catenella appeared in plankton samples at Tio Point, Tory Channel on 8 February 2010, and up until 5 May 2010 frequently occurred in weekly monitoring samples. The highest numbers were 600 cells/litre. Mouse assays carried out on Greenshell mussels from this location in February and March 2010 revealed traces of STXs, though these were just below the action level at 0.35-0.38 mg/kg STX equivalents. A solitary cell *of A. catenella* was observed at East Bay on 19 May 2010 and HPLC analysis of mussels returned a screen result of 0.25 mg/kg. In May 2006 a solitary cell of *A. catenella* was identified in a sample from Cannon Point, Port Ligar, Pelorus Sound.

1.3. Saxitoxin analogues and their toxicity

More than 20 analogues of saxitoxin have been described (Figure 3). The chemical analysis of saxitoxins is complicated by the number of molecular forms in which they are produced by the dinoflagellate and further biochemically transformed after ingestion by shellfish. An additional complication is that each molecular form has a different specific toxicity.

Saxitoxins



STX = Saxitoxin
NEO = Neosaxitoxin
GTX = Gonyautoxins
dc = Decarbamoyl

	R_1	R_2	R_3	R_4	R_5
STX	H	H	H	$CONH_2$	OH
GTX2	H	H	OSO_3^-	$CONH_2$	OH
GTX3	H	OSO_3^-	H	$CONH_2$	OH
NEO	OH	H	H	$CONH_2$	OH
GTX1	OH	H	OSO_3^-	$CONH_2$	OH
GTX4	OH	OSO_3^-	H	$CONH_2$	OH
GTX5 (B1)	H	H	H	$CONHSO_3^-$	OH
C1	H	H	OSO_3^-	$CONHSO_3^-$	OH
C2	H	OSO_3^-	H	$CONHSO_3^-$	OH
GTX6 (B2)	OH	H	H	$CONHSO_3^-$	OH
C3	OH	H	OSO_3^-	$CONHSO_3^-$	OH
C4	OH	OSO_3^-	H	$CONHSO_3^-$	OH
dcSTX	H	H	H	H	OH
dcGTX2	H	H	OSO_3^-	H	OH
dcGTX3	H	OSO_3^-	H	H	OH
dcNEO	OH	H	H	H	OH
dcGTX1	OH	H	OSO_3^-	H	OH
dcGTX4	OH	OSO_3^-	H	H	OH

Figure 3. Molecular structures of known saxitoxin analogues

Table 1. Relative toxicities of different STX analogues produced by *Alexandrium catenella*

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

Table 1. shows the relative toxicities of the different saxitoxin analogues that are produced by *Alexandrium catenella*. Saxitoxin (STX) is the parent compound and is the most toxic, so it has a value of 1. The toxicity of the other compounds vary. For example GTX1 and neo STX have similar levels of toxicity to STX, while C1 and C2 respectively are only 1/100 and 1/10 as toxic as STX. Therefore although C1 and C2 may be abundant in the algae and shellfish they make only a minor contribution to the total toxicity.

An effect based assay, such as the MBA, provides in one step an approximate measure of the total toxicity of the sample. Estimation of the total toxicity of a sample by chemical analysis requires the quantification of each individual molecular type, calculation of the 'toxicity' represented by this amount, and summing of their individual contributions to arrive at an estimate of total toxicity.

1.4. The STX screen test

The chemical analysis of STXs is technically difficult because they do not naturally have the molecular characteristics necessary to strongly absorb, or emit by fluorescence, specific wavelengths of light. This means that although the various toxins can been separated on a chromatography column they cannot be easily detected. To overcome this, they need to be chemically converted (oxidised) into a form which becomes fluorescent under UV light and enables them to be to be measured at very low concentrations. There are two ways of doing

this; either the toxins in a sample are oxidised before or after separation on the chromatography column. The pre-column oxidation method (commonly referred to as the Lawrence method) was chosen (Holland et al. 2010a) because of its sensitivity and reliability. Also it only requires a single 6 minute run (using an ultra high performance LC) and can be accomplished at a sustainable price. The down side with the pre-column oxidation method is that several of the toxins (e.g. GTX1,4 and C3,C4), which have very different specific toxicities (refer to Table), are converted into the same co-eluting fluorescent compounds and so cannot be distinguished. In this situation the most conservative case is assumed (i.e. the peak entirely represents the most toxic compound) and used in the summation of the total toxicity. This is the reason why the STX screen analysis is invariably an overestimate of the real value.

The screen method has been thoroughly validated and shows excellent precision (repeatability and reproducibility) on a variety of shellfish samples and comparison with MBA results is good (Holland et al. 2010b). Using the screen method the limits of detection for STXs in Greenshell mussels is in the range of 2-13 µg/kg, at least 30-200 times lower than the action level of <0.4 mg/kg.

1.5. The STX confirmation test

To definitively determine the concentrations of the individual STX analogues in shellfish, a more complex version of the Lawrence method (Lawrence and Niedzwiadek, 2001) is used. This involves the use of an ion-exchange fractionation step, two oxidizing agents (periodate and peroxide), additional UPLC steps and manual interpretation of the data. This makes this test prohibitively laborious and expensive for routine screening purposes, but results in determination of all individual STX components (including the ambiguous lower toxicity forms) and accurate quantification of total toxicity.

1.6. Closure criteria based on the STX screen test

Not Detected. No toxin peaks above their respective limits of detection (LOD). No regulatory action.
<0.1-0.4 mg/kg total STX equiv. Sum of compounds present over the total STX reporting threshold. Toxins detected but no risk of the 0.8 mg/kg total STX equiv. regulatory limit being exceeded. No regulatory action but a signal for more surveillance.
≥0.4 mg/kg total STX equiv. Toxins detected with potential for levels to be close to or above the regulatory limit. This result requires either running of a full confirmatory test or closure of the harvest area.

The screen test results that are issued are <0.1mg/kg (clear); 0.1-04 mg/kg (detection below the regulatory limit); ≥0.4 mg/kg (confirmation test or closure).

1.7. Reopening criteria

The current reopening criteria include two consecutive confirmation tests <0.8 mg/kg or two consecutive screen tests <0.4 mg/kg. A sample returning a screen test ≥ 0.4 mg/kg (even if <0.8 mg/kg) requires a confirmation test with a result of <0.8 mg/kg to ensure it is below the regulatory limit. Spatial and depth profile sampling is also required for reopening. Based on the current re-opening criteria, if after an area is reopened a routine screen result of >0.4 mg/kg is obtained, a further confirmatory test is required, even in the absence of any supporting evidence (i.e. phytoplankton) of renewed contamination.

1.8. Sampling and analysis

Phytoplankton samples were routinely collected from monitoring sites using a 15m Lund tube, preserved with Lugol's iodine and cells counted under an inverted microscope. On a few occasions various depths in the water column were sampled using van Dorn bottle. All phytoplankton analyses were carried out at the Cawthron Institute. Most of the shellfish toxin analyses were carried out for the Marlborough Shellfish Quality programme (MSQP) by the Cawthron Institute. A few samples (<20) were collected for the Marlborough District Health Board as part of their recreational shellfish safety programme and analysed by AsureQuality, Auckland.

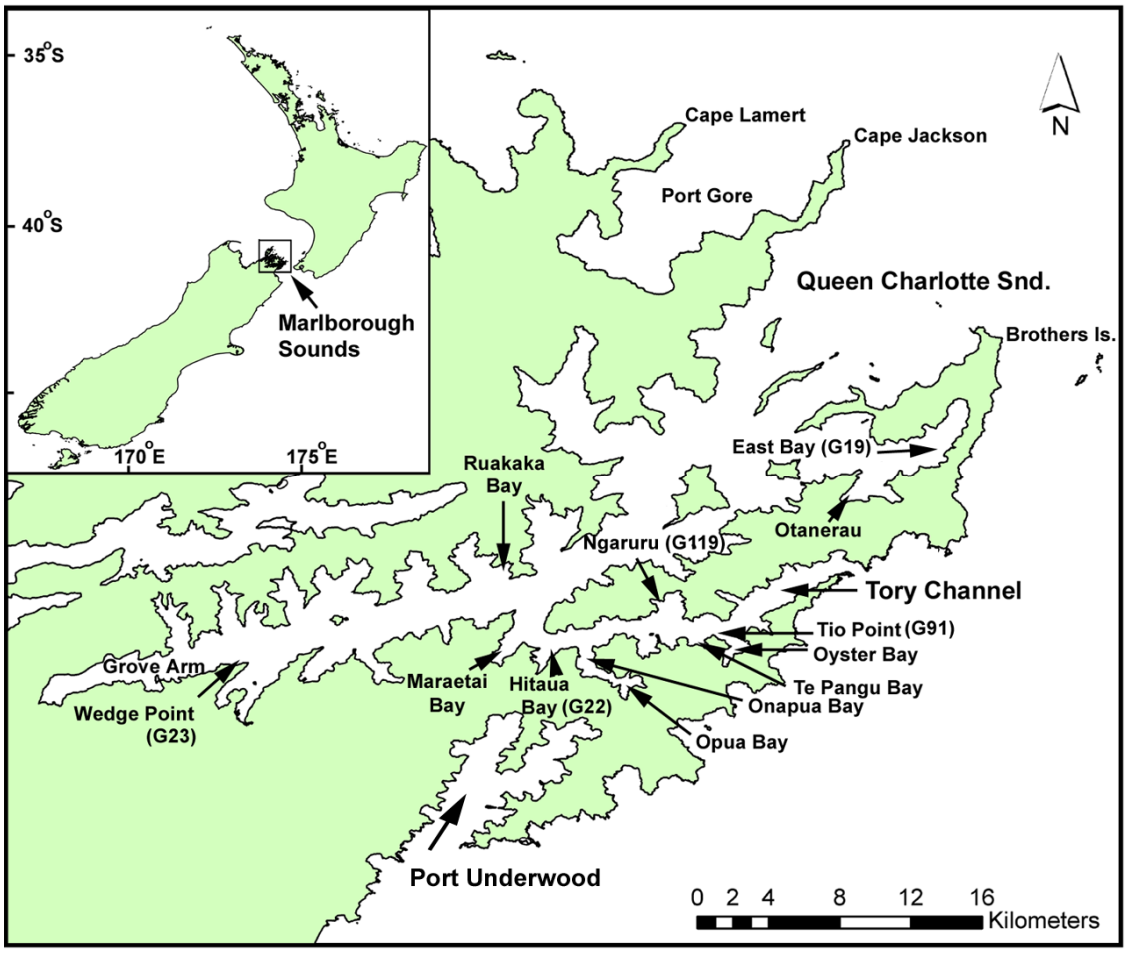


Figure 4. Sampling locations in Queen Charlotte Sound.

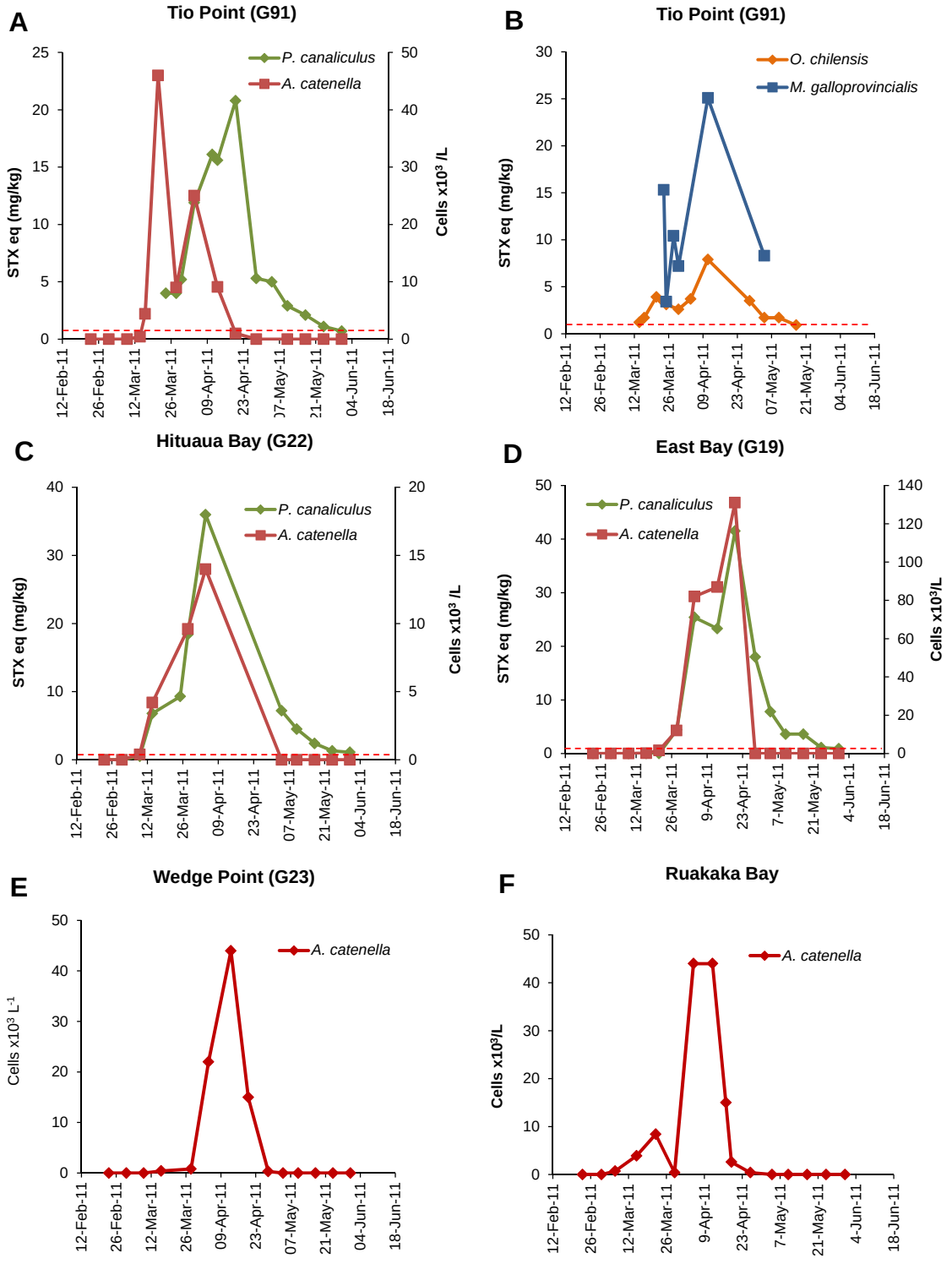


Figure 5. *Alexandrium catenella* cell numbers (cells x10^3 / L) in 15m integrated water column samples and levels of PSP-toxins (mg/kg STX equivalents) determined by the screen test in several bivalve species (*Perna canaliculus, Ostrea chilensis, Mytilus galloprovincialis*) at Queen Charlotte Sound monitoring locations, Feb-June 2011.

2. PROGRESSION OF THE BLOOM AND SHELLFISH TOXICITY IN QUEEN CHARLOTTE SOUND

The first *A. catenella* cells were seen in a sample from Ruakaka Bay on 7 March 2011, and on 9 March at Hitaua Bay, where a screen test of Greenshell mussels returned a screen test of 0.7 mg/kg STX equivalents (Figs 4, 5 & 6). Samples collected at Wedge Point, Tio Point and East Bay on 9 March contained no cells nor did mussels from the latter two sites return positive STX screen tests. The next week (14 March) cell numbers at Hitaua Bay had increased substantially (4.2 $x10^3$ cells/L) as had STX levels (6.8 mg/kg STX equiv) a few cells were observed at Tio Point along with 1.2 mg/kg STX equiv in oysters. Within that week the bloom dispersed widely and a few cells were observed at Wedge Point and East Bay on 16 March. From this point on cells numbers and STX screen levels increased at all sites, peaking at Hitaua Bay on 4 April and Tio Point and East Bay on 20 April.

Only two samples collected during the course of the public health recreational shellfish monitoring programme tested positive. These were Greenshell mussels (0.87 mg/kg STX equiv) from Wedge Point collected on 21st March and blue mussels (0.55mg/kg STX equiv) from Ships Cove collected on 28th March.

After 20 April cells rapidly disappeared and toxin levels began to decline. There was a hiatus in the sampling at Hitaua Bay between 4 April and 4 May so there is no data available for that period from this site. After 20 April all cells disappeared from East Bay and Tio Point and only a few remained at Wedge Point and Ruakaka Bay. The rapid disappearance of *A. catenella* coincided with a north-west storm over Easter weekend (22-25 April).

Maximum total STX by the screen test was 41.5 mg/kg STX equiv at East Bay (G19) on 20 April. Blue mussels reached a maximum of 25 mg/kg at Tio Point on 11 April compared to a level of 16.1 mg/kg STX equiv in Greenshell mussels at this time. Flat oysters did not accumulate STXs to the same extent as Greenshell and blue mussels and only reached a maximum of 7.9 mg/kg STX equiv on 11 April at Tio Point.

On 25 March more detailed sampling of Tory Channel revealed that *A. catenella* was widespread (Figure 6) and high cell numbers (6.3 $x10^5$ cells/L) were encountered in the inland reaches of Opua Bay in association with a visible red tide (Figure7). Opua Bay was visited on two more occasions (30 March, and 12 April) to collect bulk quantities of algal material for toxin purification and very high numbers of cells (> 4.0 $x10^6$ cells/L) persisted throughout this period.

Table 2. Shellfish harvesting closure and opening dates in commercial and recreational areas of Queen Charlotte Sound

Site code and location	Growing area code	Close	Open	Duration (days)
G22 Tory Channel	1521A	16 March-11	20 June -11	97
G91 Oyster Bay	1521	23 March-11	17 May-11	56
G19 East Bay	1520	01 April-11	20 June-11	81
G119 Ngaruru Bay	1521-6/F	16 March-11	25 May-11	71
MDHB* closure	All QS Sound.	25 March-11	21 June-11	89

*Marlborough District Health Board

On 20 April a few cells of *A. catenella* (0.1-0.2 x10^3 cells/L) occurred in samples from the eastern side of Cape Jackson (Anakakata Bay) and within Port Gore (Waimetete Bay), and on 28 April a single cell was seen at Opihi Bay Port Underwood. These were the most distant observations made (Figure 6).

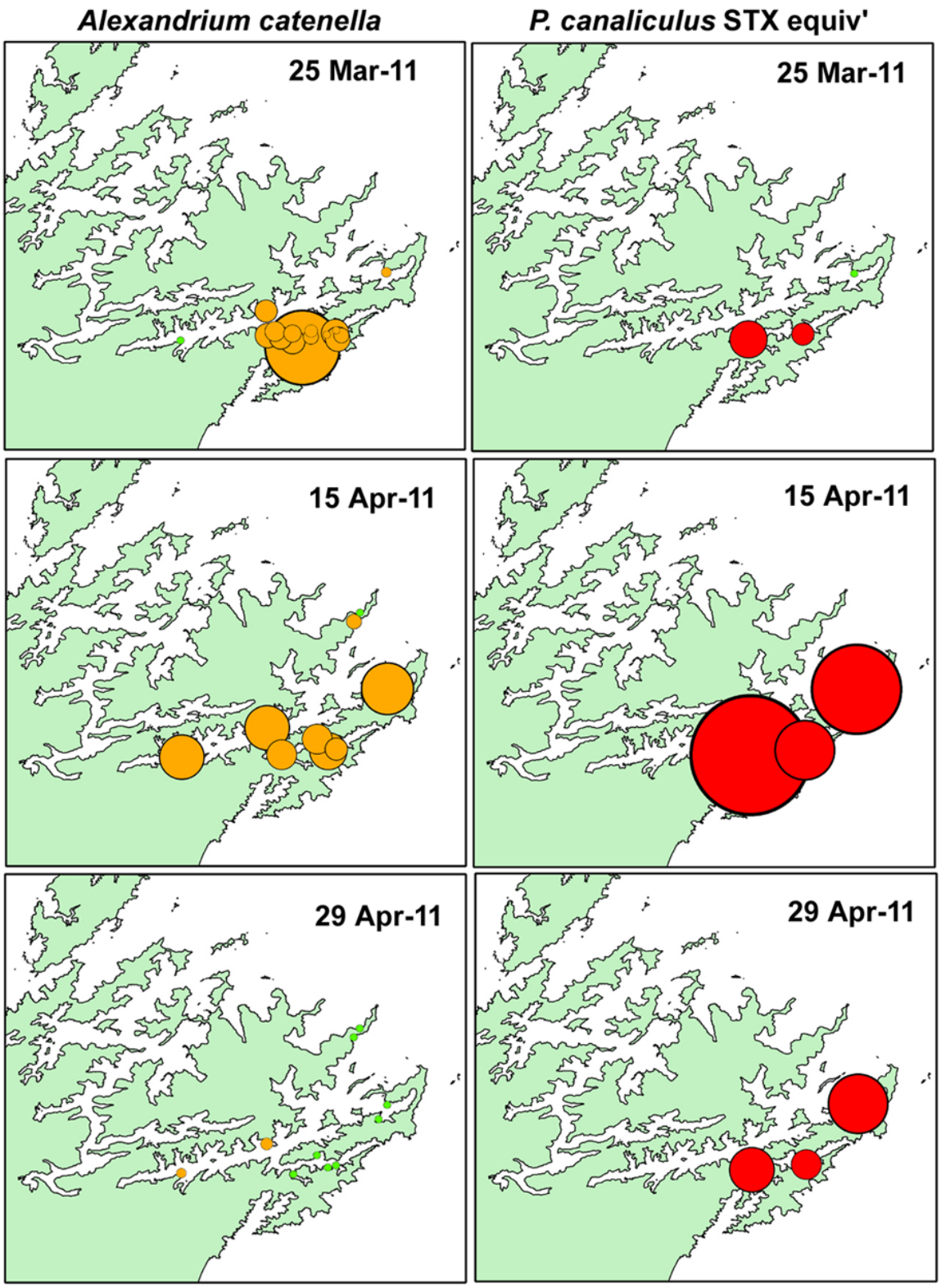


Figure 6. Distribution of *Alexandrium catenella* cells and toxicity of Greenshell mussels (*Perna. canaliculus*) during early (25 March 2011), mid (15 April 2011) and late (29 April 2011) phases of the bloom.

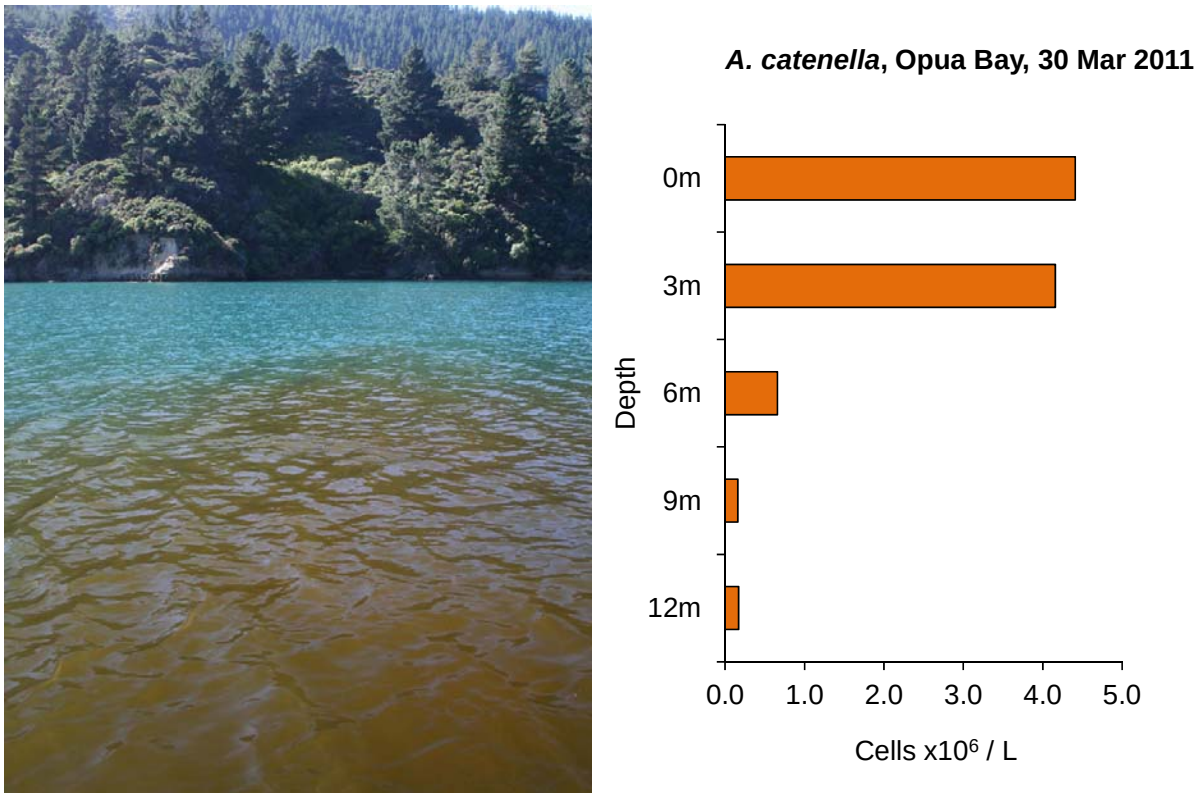


Figure 7. "Red tide' bloom of *Alexandrium catenella* in Opua Bay and cell numbers in this patch of discoloured water.

2.1. Water column conditions during the bloom

CTD-fluor (conductivity/temperature/depth/chlorophyll fluoresence) profiles (Figure 8) measured on 25 March 2011, showed that within Tory Chanel itself the water column was uniformly mixed (at least in the top 25m) with a temperature of 14.7 ^{o}C and salinity of 34.6 psu. Uncorrected chlorophyll *a* (Chl*a*) concentrations were low at 0.3 µg/L. In Opua and Oyster Bays temperatures were 0.2-0.5 ^{o}C higher than in the main channel at around 15.0 -15.2 ^{o}C. The water column within these bays was very weakly thermally stratified. Salinity (~34.7) was about 0.1 psu higher than in the main channel and was uniform from top to bottom. High chlorophyll a concentrations existed in the bays associated with the high cell numbers (> 4.0 x10^{6} cells L^{-1}) of *A. catenella* (Figure7). In both bays the phytoplankton biomass was highly stratified. In Opua Bay the majority of the biomass was in the top 4 metres with Chl*a* concentrations up to 4.0 µg/L, while in Oyster Bay there was a subsurface Chl *a* maximum (~3.5 µg/L) between 5 and 9 metres (Figure 8).

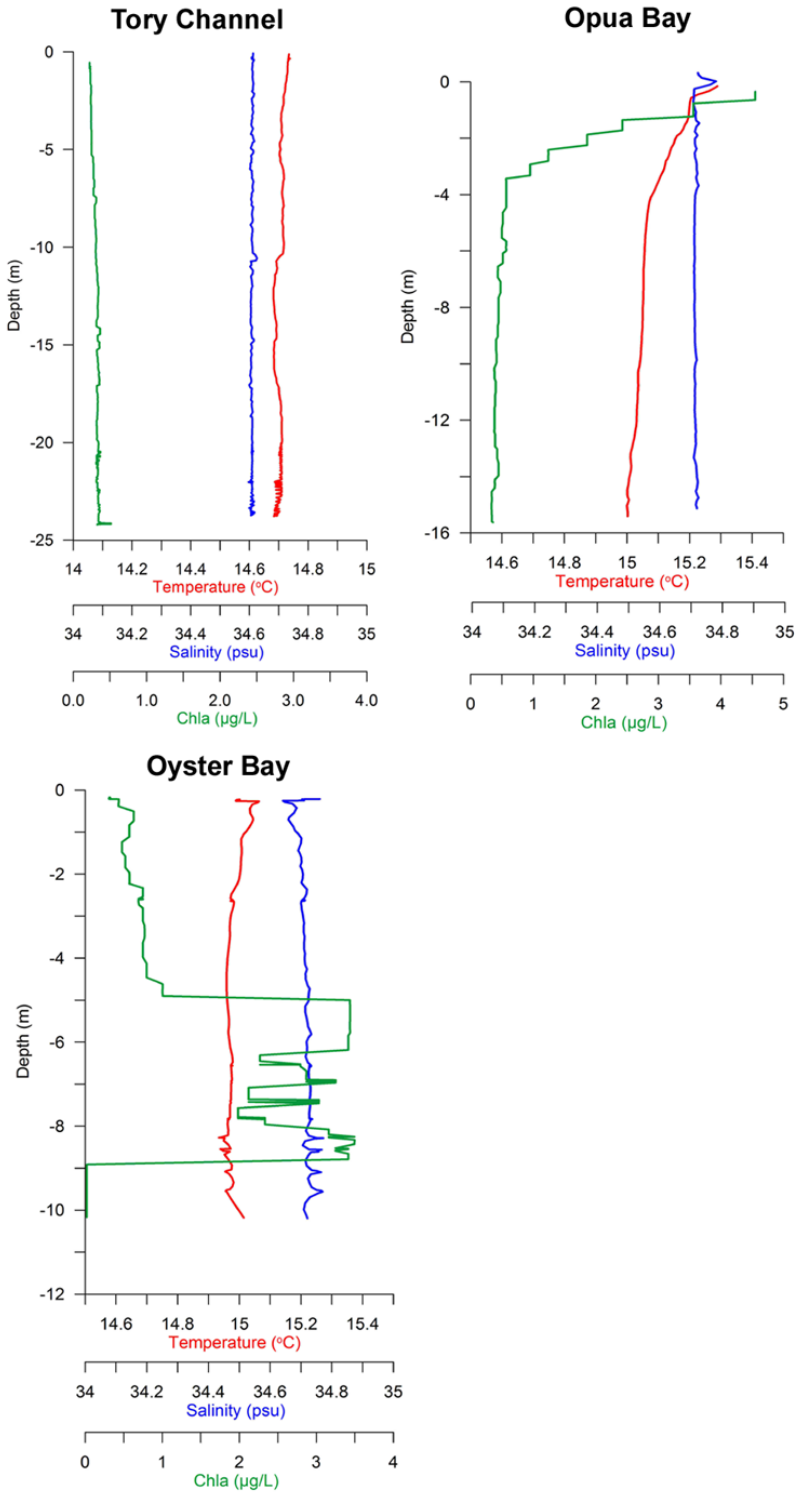


Figure 8. CTD profiles of temperature, salinity and chlorophyll a (Chla) concentrations in Tory Channel and embayments 25 March 2011.

2.2. Detection of *A. catenella* in seawater samples using a qPCR analysis

The presence of *Alexandrium catenella* in the water samples provided the opportunity to trial a newly developed quantitative polymerase chain reaction (qPCR) assay for the semi-automated enumeration of cell numbers in a real bloom situation. This technique has the potential to supplement manual microscopic counts and the ability to detect cells at very low densities.

Sample collection and DNA extraction

Sea water samples (25 mL) for qPCR analysis were collected and preserved immediately with the addition of 25 mL RNAlater® in the field. Subsequently the DNA in the samples was extracted and the assays run in the lab.

Results

There was a good correlation ($R^2 = 0.91$) over a wide range of cell abundances (from 5 $x10^2$ – 4 $x10^6$ cells L^{-1}) between cell counts of *A. catenella* and the results of the cycle threshold of the qPCR assay (Figure 9).

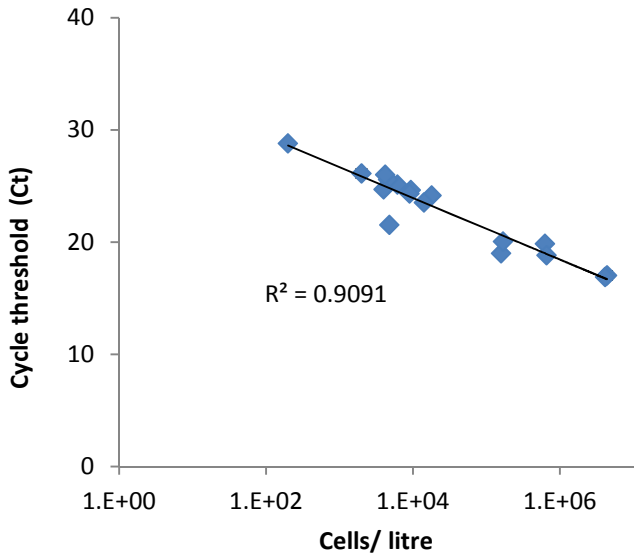


Figure 9. Quantitative PCR efficiencies of probes and primers, tested on environmental DNA samples plotted against corresponding light microscope cell counts of *A. catenella*. Curves show cycle threshold values (Ct value) plotted over the log transformed cell equivalents. Error bars represent standard deviations of two replicates.

The cycle threshold is the number of PCR cycles that have to run before cells are detected, so the higher the number of cycles the lower the number of cells. These results show that this assay has potential as a useful tool for monitoring *A. catenella* cell abundance during bloom events as well as for the detection of cells at low abundance. In these analyses, 5 cells was the lowest actual number of cells in the 25 mL seawater samples collected.

Theoretically a single cell can be detected and sensitivity increased by using larger sample sizes. Numerous samples can be analyzed simultaneously using the qPCR assay, and its extreme sensitivity should enable the detection of the dinoflagellate at a very early stage of bloom development.

2.3. *Alexandrium catenella* resting cysts

Alexandrium catenella has a sexual phase in its life cycle that results in the formation of immotile resting cysts (hyponzygotes) that can persist in seafloor sediments for months to years. The cysts enable survival of the dinoflagellate when environmental conditions are unfavourable to motile cells. They are a mechanism for dispersal and genetic recombination, and provide a dormant seed source that can lead to the generation of new blooms. Simultaneous mass germination of cysts can lead to the sudden development of *Alexandrium* spp. blooms, unlike blooms of some other toxic species that may take some time to develop.

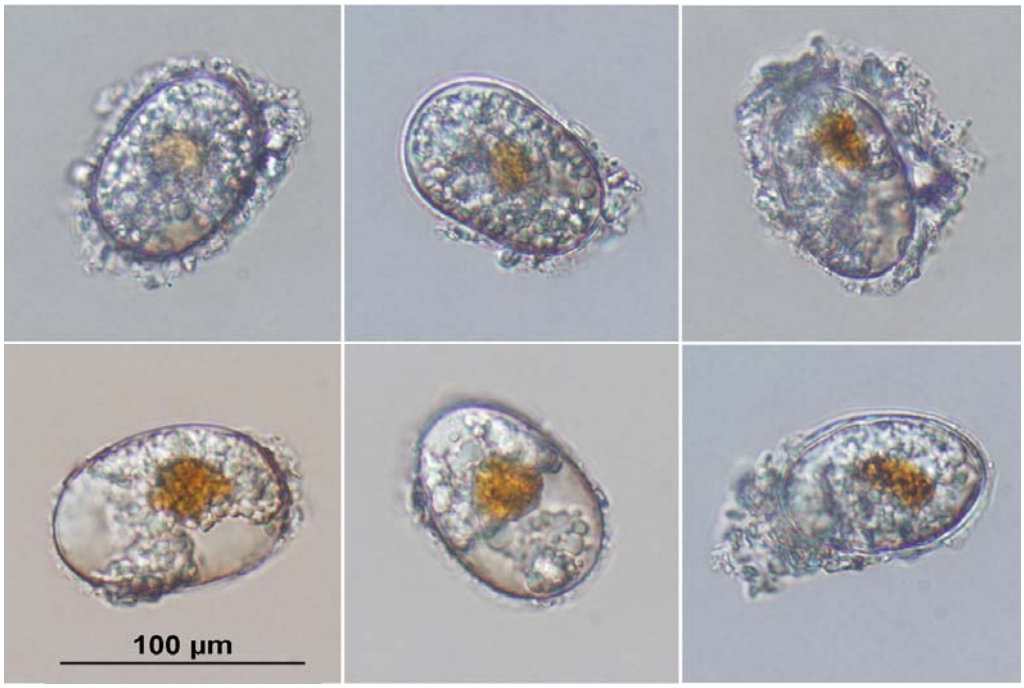


Figure 10. *Alexandrium catenella* resting cysts from Opua Bay sediments.

Sediment samples were collected with a van Veen grab sampler from a number of locations around Queen Charlotte Sound on 21 June 2011 (Table 3). Three 6cm diameter cores were collected from each grab sample, the top 1cm was sliced off and made into a composite sample. Homogenous sub-samples (5mL) were sonicated and coarse inorganic particles removed by sedimentation with 4x washes with 500mL of filtered seawater. Cysts (Figure 10) were collected on a 20 µm mesh screen for identification and counting.

Alexandrium catenella cysts were observed in all but one of the sediment samples collected from Queen Charlotte Sound (Table 3). At most sites the numbers were rather low and it is questionable whether these cyst numbers are sufficient to initiate new blooms. However high cysts numbers were found in Opua Bay (up to 9.1 x10^5 cysts/m^2) where very high numbers of motile cells persisted during the bloom (Figure 11). It is likely that this seed source will lead to the generation of future blooms at this location, which may then spread throughout the sound. It is recommended that Opua Bay be made an additional routine plankton monitoring site to provide an early warning of *A. catenella* bloom development.

Table 3. Counts of *Alexandrium catenella* resting cysts in Queen Charlotte Sound sediments

Location	cysts/ml	cysts/g wet wt.	cysts x10^3/m^2
Grove Arm-1 (off Ngakuta Bay)	0.4	0.3	4.2
Grove Arm-2 (off Wedge Point)	1.2	1.0	16.5
Ruakaka Bay (A)	nd	nd	nd
Ruakaka Bay (B)	1.2	0.9	16.5
Hitaua Bay	0.8	0.5	6.5
Onepua Bay	7.2	4.6	62.8
Opua Bay (A)	68.0	51.7	634.1
Opua Bay (B)	68.0	51.4	692.3
Opua Bay (C)	93.6	71.3	912.6
Tio Point	1.8	1.2	16.0
Oyster Bay	0.8	0.6	5.7
Ngaruru Bay	0.8	0.5	6.5
Otanerau Bay	24.8	15.8	220.5
East Bay (A)	10.4	7.7	80.3
East Bay (B)	1.6	1.3	17.2

nd = not detected

Processing and microscopic examination of sediment samples for dinoflagellate cysts is tedious, time consuming and hence expensive. This prevents the analysis of large numbers of samples to assess bloom potential. The qPCR assay for *A. catenella* motile cells described in the previous section, may be applicable to the detection and enumeration of resting cysts in sediment samples and trials to assess the feasibility of this are planned. A similar approach has been successful in quantifying *Alexandrium* cysts off the coast in the Gulf of Maine (Erdner et al. 2010).

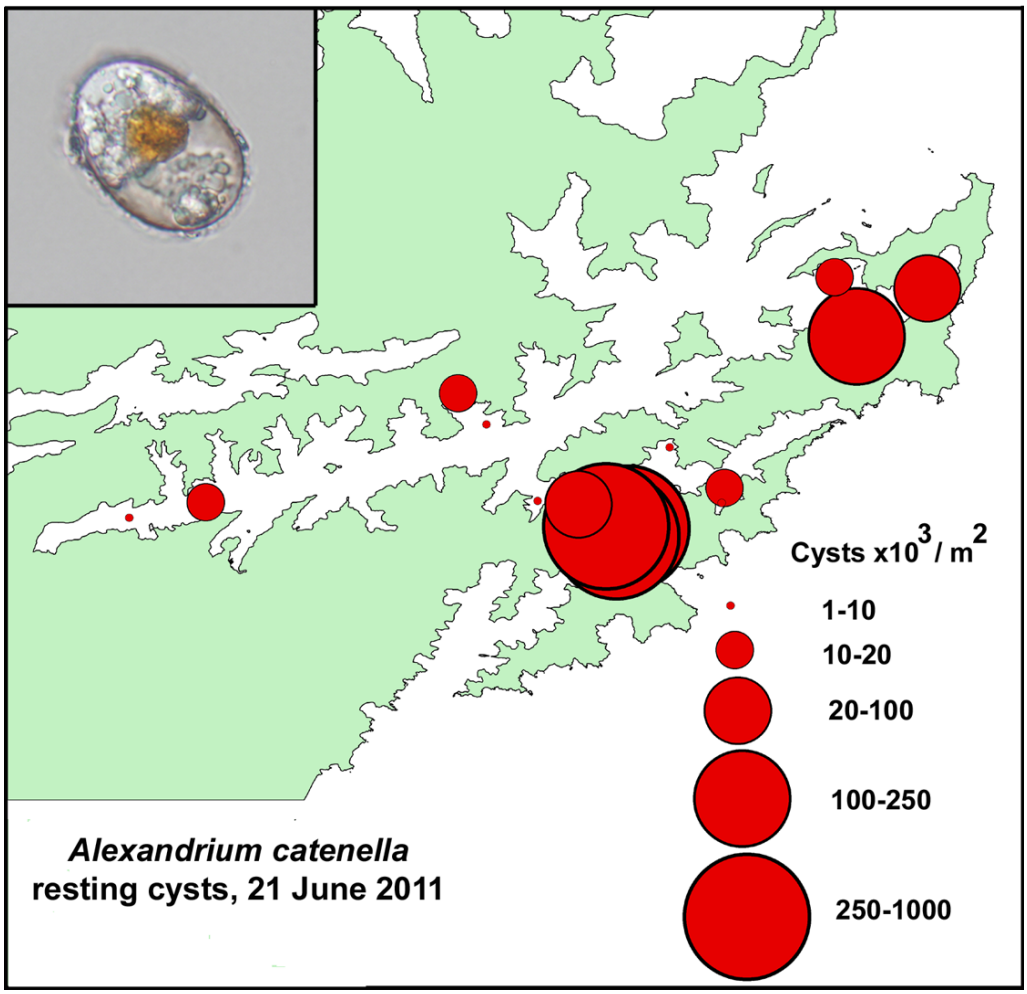


Figure 11. *Alexandrium catenella* resting cysts (cysts x10^3 / m^2) in the sediments of Queen Charlotte Sound, sampled 21 June 2011.

3. SAXITOXIN PROFILES

3.1. Toxin profiles in *Alexandrium catenella*

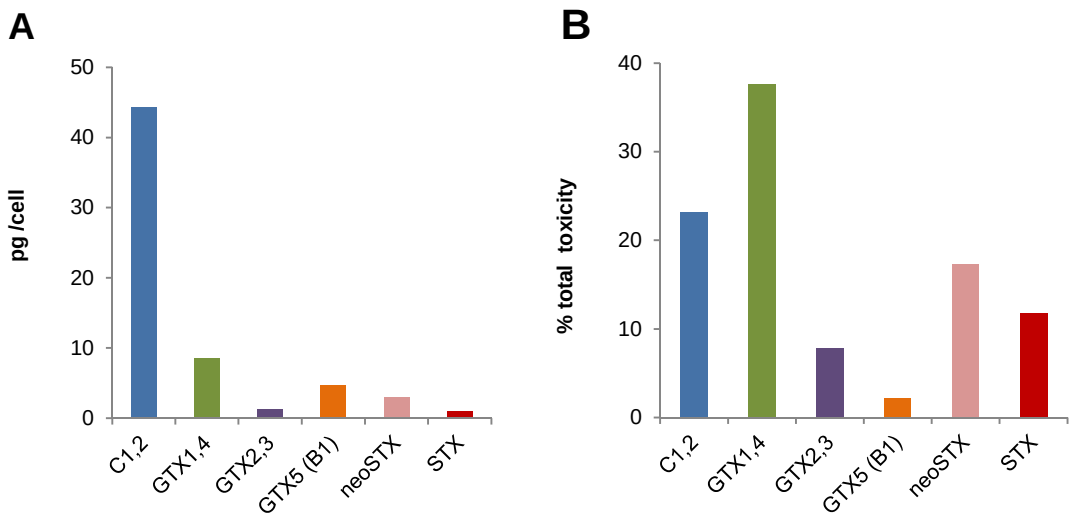


Figure 12. STX screen profiles in *Alexandrium catenella* cell concentrates collected from Opua Bay analysed using the Lawrence pre-column oxidation method. **A** Toxin content (pg/cell) of each group of analogues. **B**. Relative toxicity contributed by each group of analogues (STX equivalents/g wet wt.).

Analysis of *A. catenella* cell concentrates collected with a plankton net showed that the toxin profile, in terms of the amount of toxin per cell, was substantially dominated by low toxicity C1 and C2 analogues (Figure 12A). However, although present in much lesser amounts, the other more potent analogues (GTX-1,4, GTX-2,3, neoSTX and STX) make the major contribution to the total toxicity of the cells (Figure 12B). The toxin profile of *A. catenella* from Tory channel is very similar to that previously identified in isolates from the Bay of Plenty (MacKenzie et al. 2004).

Toxicity profiles in contaminated shellfish (Figure13) determined by the screen and conformation tests show clear affinities with the toxicity profiles of *A. catenella,* although there is some suggestion of *in vivo* toxin biotransformation of lesser (e.g. C1,2) to more toxic forms (neoSTX and STX) especially in oysters (Figure 13 C,D).

3.2. Toxin profiles in mussels and oysters

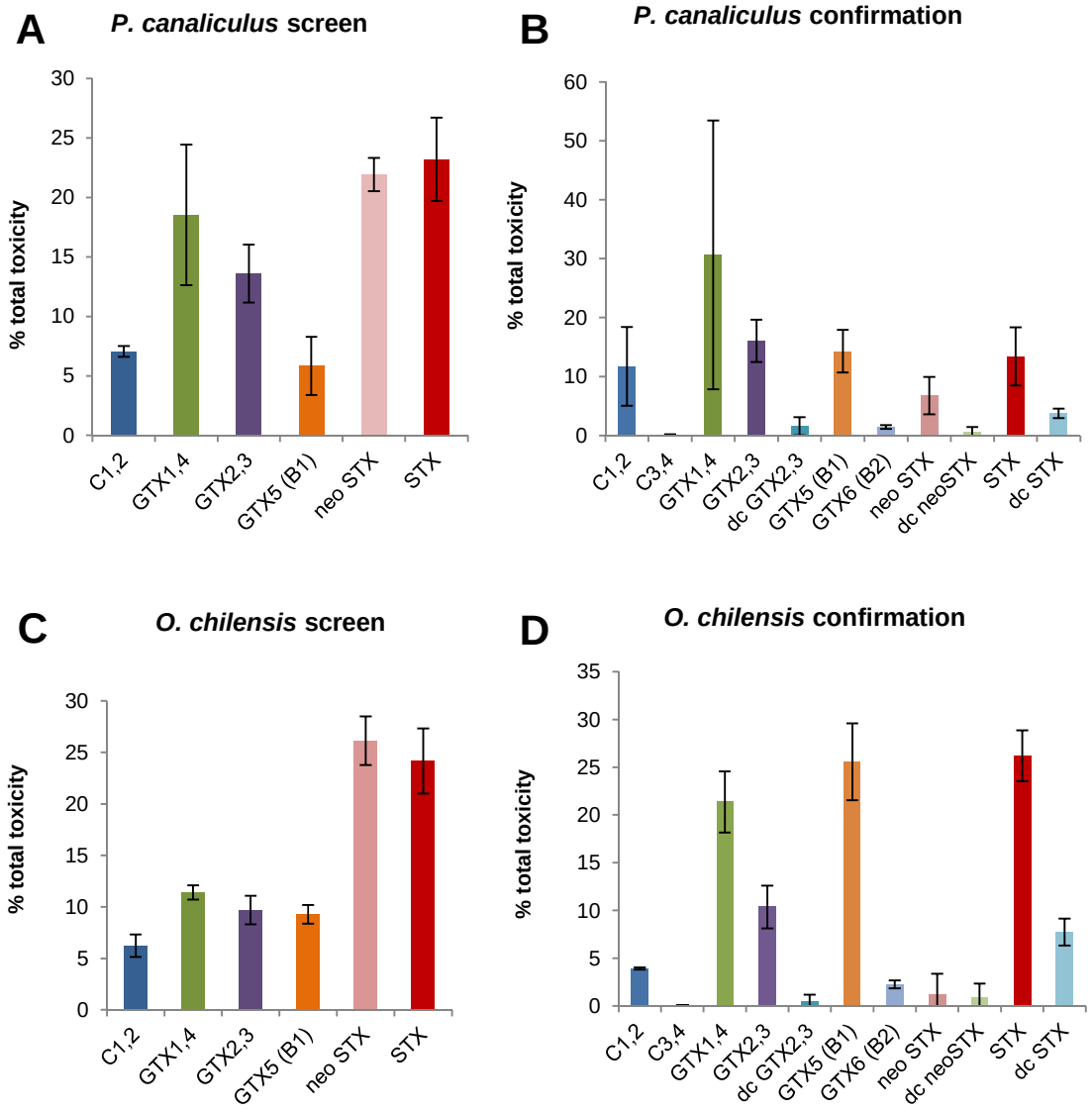


Figure 13. Screen and confirmation toxin profiles in Greenshell mussels (*Perna canaliculus*) and flat oysters (*Ostrea chilensis*) from Oyster Bay, Tory Channel (site G91) 5-17 May 2011

3.3. Screen tests versus confirmation tests

The ratio of confirm/screen for Greenshell mussels determined during the validation exercise (Holland et al. 2010b) was 0.26 ± 0.07 carried out on shellfish contaminated by *Gymnodinium catenatum* toxins. The ratio of confirm/screen for shellfish contaminated by *A. catenella* was 0.35 ± 0.08 and 0.39 ± 0.14 for oysters and Greenshell mussels respectively (Table 4).

Table 4. Total toxicity determined by screen and confirmatory tests on mussels (*Perna canaliculus*) and oysters (*Ostrea chilensis*) on the same samples.

Oysters (*Ostrea chilensis*)				
Sample site	**Sample date**	**Screen**	**Confirm**	**Confirm/Screen**
		STX eq (mg kg^{-1})		
G91	14 Mar 11	1.2	0.40	0.33
G91	16 Mar 11	1.7	0.40	0.24
G91	21 Mar 11	3.9	1.10	0.28
G91	4 May 11	1.6	0.69	0.43
G91	4 May 11	1.7	0.72	0.42
G91	10 May 11	1.7	0.56	0.32
G91	17 May 11	0.9	0.39	0.43
		Mean ± Stdev		**0.35 ± 0.08**
Mussels (*Perna canaliculus*)				
G91	4 May 11	2.9	1.10	0.38
G91	10 May 11	2.1	0.56	0.20
G91	17 May 11	1.1	0.25	0.22
G91	31 May 11	0.7	0.22	0.31
G91	13 Jun 11	0.6	0.26	0.43
G22	9 Mar 11	0.7	0.50	0.71
G22	14 Mar 11	6.8	2.30	0.34
G22	17 May 11	2.4	0.91	0.38
G22	7 Jun 11	0.8	0.22	0.28
G22	13 Jun 11	0.7	0.25	0.36
G22	13 Jun 11	0.8	0.25	0.31
G22	13 Jun 11	0.7	0.23	0.33
G19	28 Mar 11	4.4	2.10	0.47
G19	31 May 11	0.9	0.62	0.68
G19	7 Jun11	0.5	0.09	0.18
G19	13 Jun 11	0.7	0.3	0.43
G19	13 Jun 11	0.5	0.25	0.50
G19	13 Jun 11	0.6	0.3	0.50
		Mean ± Stdev		**0.39 ± 0.14**

The reopening criteria require that replicate spatial (e.g. several depths) sampling and analysis is carried out to ensure that samples constitute a fair representation of the population. Table 5 shows the variation between multiple samples analysed to date. In general the replication is good and makes little difference to re-opening decisions.

Table 5. Variation between screen tests of replicate samples collected from culture lines

Site	Date	Species	Location	STX mg/kg	Mean ± stdev	% variation
Tio Point	4/5/11	Flat oyster	Top	1.6		
		Flat oyster	Bottom	1.7	1.6 ± 0.1	4.3
Tio Point	4/5/11	Greenshell mussel	Top	1.9		
		Greenshell mussel	Middle	2.9	2.4 ± 0.7	29.5
Hitaua Bay	4/5/11	Greenshell mussel		6.3		
		Greenshell mussel		5.9		
		Greenshell mussel		7.2	6.5 ± 0.1	10.3
Hitaua Bay	13/6/11	Greenshell mussel		0.7		
		Greenshell mussel		0.8	0.7 ± 0.1	7.9
		Greenshell mussel		0.7		
East Bay	4/5/11	Greenshell mussel	Top	7.8		
		Greenshell mussel	Middle	7.1		
		Greenshell mussel	Bottom	5.9	6.9 ± 1.0	13.9
East Bay	13/6/11	Greenshell mussel		0.7		
		Greenshell mussel		0.5		
		Greenshell mussel		0.6	0.6 ± 0.1	16.7

In all shellfish species analyzed (Figure 14) the majority of the toxins were contained within the digestive gland (hepatopancreas).

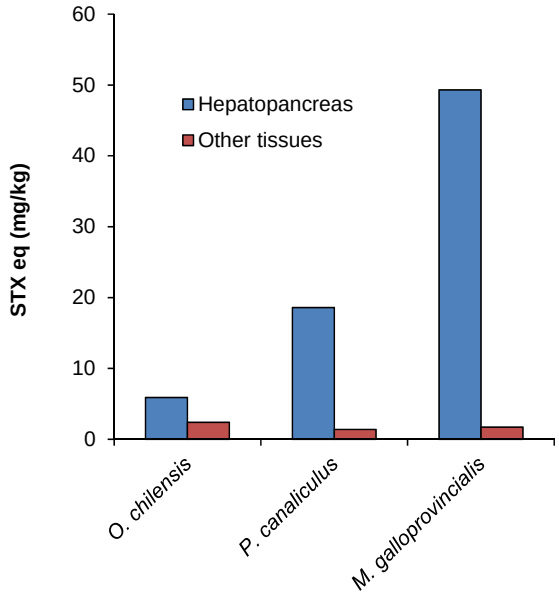


Figure 14. Distribution of STXs in shellfish tissues

4. SHELLFISH DEPURATION

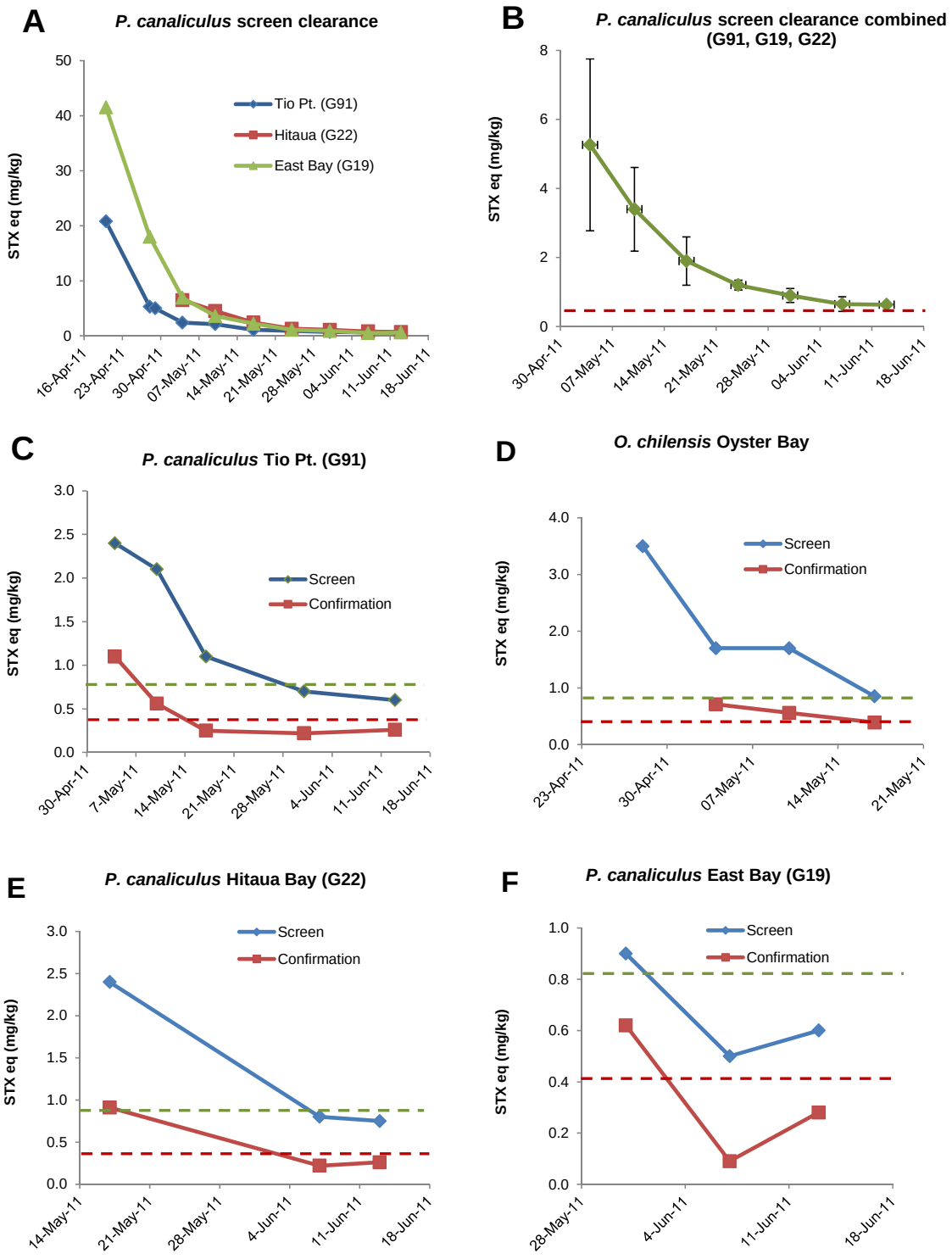


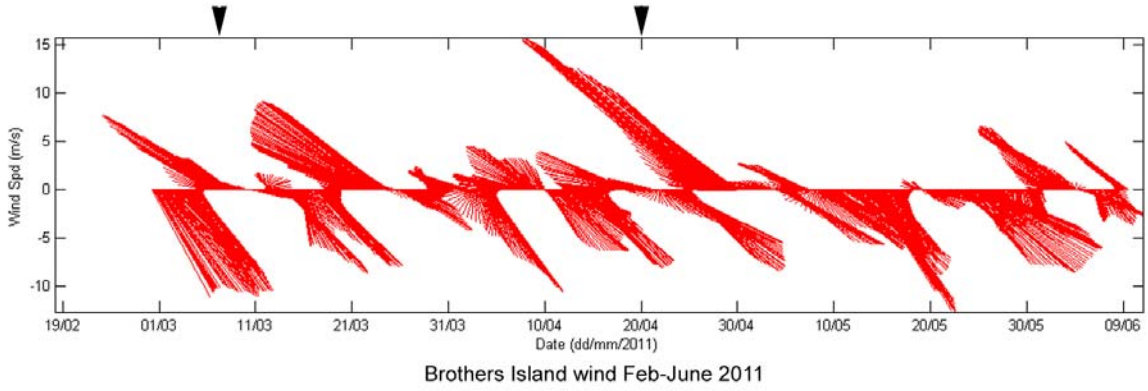
Figure 15. Clearance of STXs from Greenshell mussels and oysters. The green line indicates the confirmation action level of 0.8 mg/kg STX equ', the red line the 0.4 mg/kg STX equ' level.

During biotoxin contamination events the clearance phase after the bloom itself has crashed can substantially extend closure periods (Figure 15). The flattening out of the depuration curve as it approaches the 0.4 mg/kg STX equivalent means that the overestimation of toxicity by the screen test can unduly extend the closure period unless confirmation analyses are carried out. The confirm/screen ratios (Table 4) provide a good rule of thumb for estimating from the screen test results when is an appropriate time to begin confirmation testing. The data illustrated in Fig15 indicate that it is unnecessary to require additional confirmation tests, when subsequent screen tests return levels > 0.4 mg/kg STX equivalents during this late depuration phase. As long as screen test results are below 0.8 mg/kg and are remaining constant or continuing a downward trend, there is a good degree of certainty that the real toxicity will be below the 0.4 mg/kg STX equivalents level.

5. ENVIRONMENTAL CONDITIONS PRIOR TO AND DURING THE BLOOM

5.1. Weather records

A



B

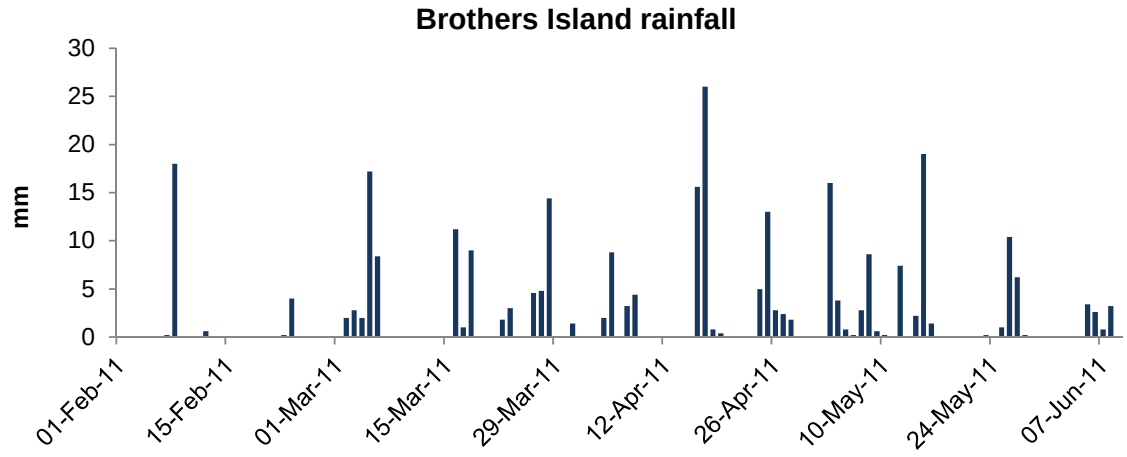


Figure 16. Weather data from the Brothers Island, February-June 2011. **A**: Wind speed and direction, the arrow heads mark the appearance and disappearance of *Alexandrium.catenella* at Tio Point, Tory Channel. **B:** Rainfall (mm).

During March, April and May 2011 the southern oscillation index was in a positive (La Nina) phase, a condition that is supposedly not conducive to upwelling and nutrient enrichment of the outer sounds (Zeldis et al. 2008) due to the preponderance of south easterly winds under this condition. In fact winds were light and alternatively from both north westerly and south easterly directions preceding and during the bloom period (Figure 16). Strong north-westerly winds over Easter weekend (22-25 April 2011) coincided with the disappearance of *A. catenella* cells from the water column throughout the sound and it seems likely that this helped bring about the demise of the bloom by inducing water column mixing and driving high current flows, although cell numbers were in decline at some sites prior to this event. There were several minor rainfall episodes prior to and during the bloom but it is unlikely these played any significant role in stimulating dinoflagellate growth.

5.2. Seawater temperatures

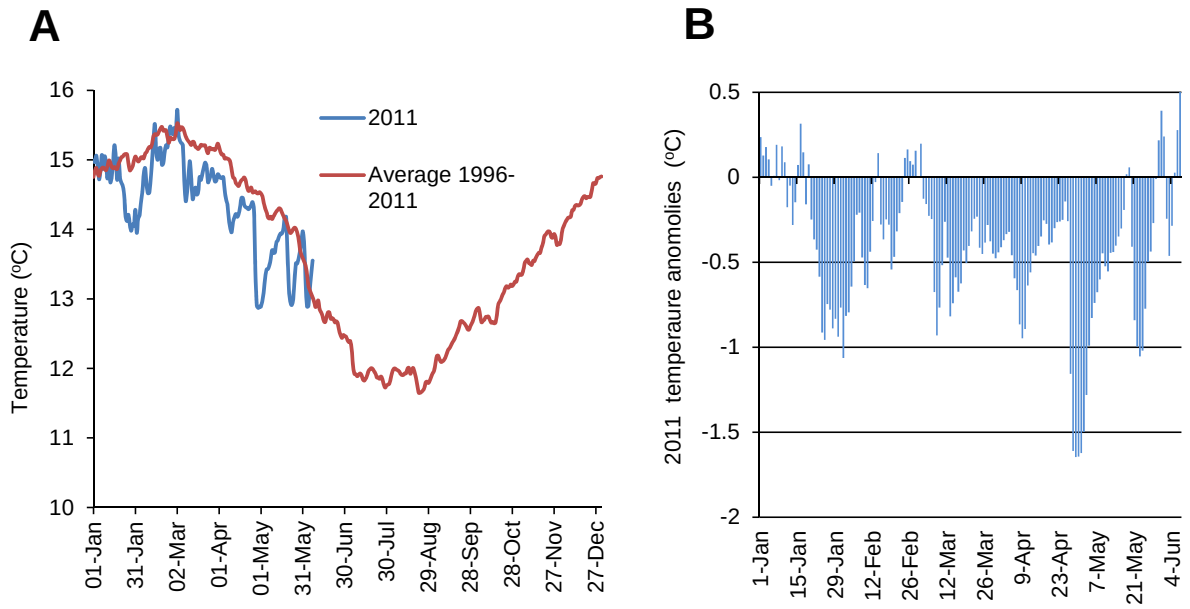


Figure 17. Seawater temperatures at the NZ King Salmon Co farm at Te Pangu Bay, Tory Channel. **A.** Temperatures measured at 5 meters depth **B.** Temperature deviation from the average.

Sea water temperatures in Tory Channel were lower by about 0.5-1.0 ºC than the average over most of the bloom period (Figure 17). At the time the first *A. catenella* cells appeared (7th-9th March) at Ruakaka and Hitaua Bays a rapid decrease in temperature from 15.2 ºC to 14.4 ºC was recorded at Te Pangu Bay. From then on, for the duration of the bloom, temperatures remained lower than average until on the weekend 22-25th April there was a further rapid decrease in temperature associated with the north westerly gale that finally brought it to an end. Water temperatures in Tory Channel are generally lower than in other parts of the sounds because it receives water directly from Cook Strait where upwelling of cool nutrient enriched water is common along the northern coastlines of the sounds. This phenomenon can be seen in satellite images of sea surface temperatures over the duration of the bloom (Figure 18).

Cool water (15.0-15.5 ^{o}C) existed for most of this time in the vicinity of Tory Channel entrance. It is possible that this upwelled water may have played some role in fuelling the growth of *A. catenella* in the Tory Chanel embayments, however the water column conditions that existed prior to or during the bloom are not regarded as unusual.

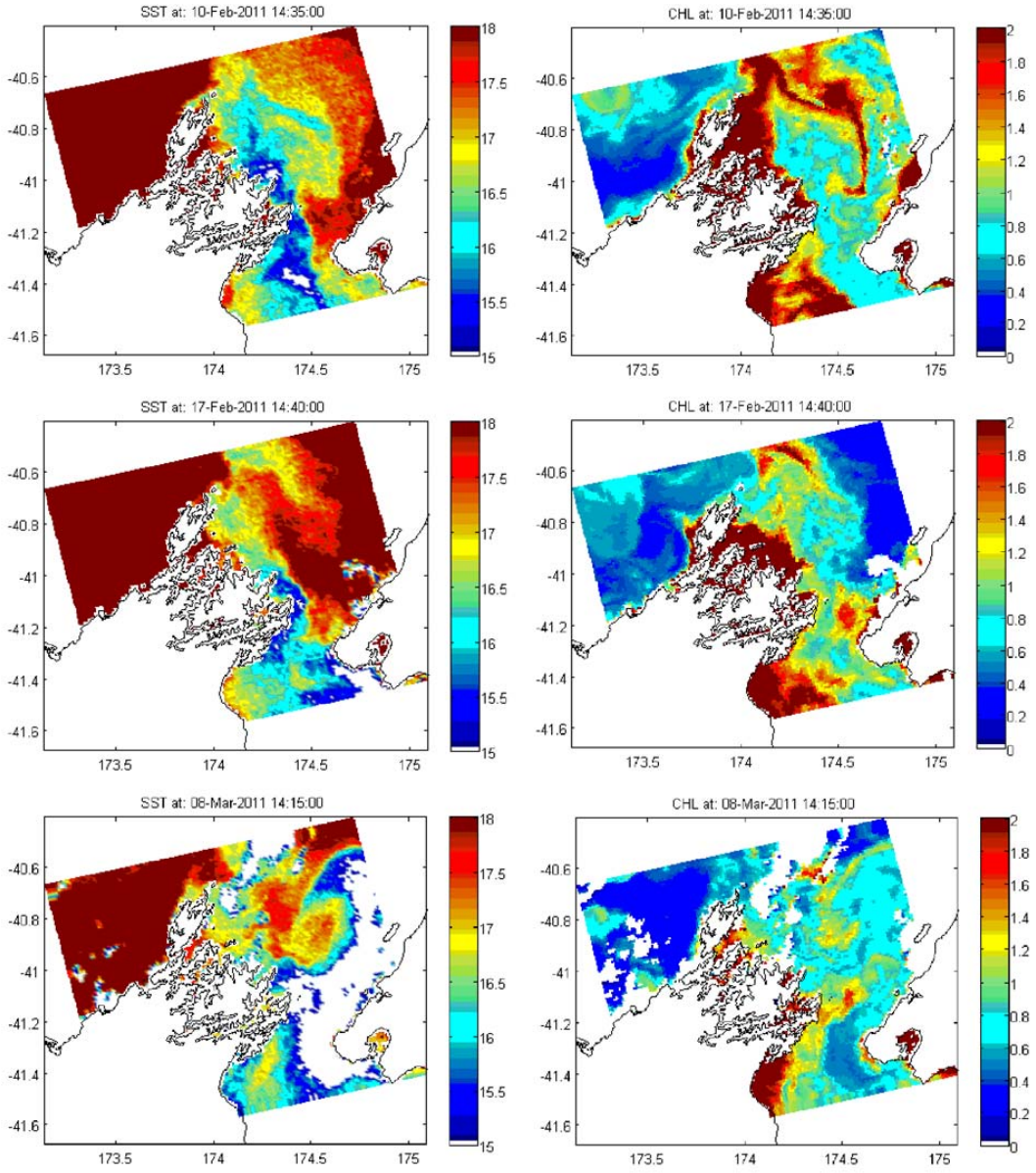


Figure 18. Satellite images of sea surface temperature and chlorophyll in the Cook Strait region, 10 Feb - 08 March 2011.

The satellite images of sea surface chlorophyll (CHL) concentrations, show regions of elevated phytoplankton biomass associated with eddies within Cook Strait to the east of D'Urville Island. These eddies are a common feature of this region associated with the he D'Urville current and are unlikely to be associated the *A. catenella* bloom in Queen Charlotte Sound.

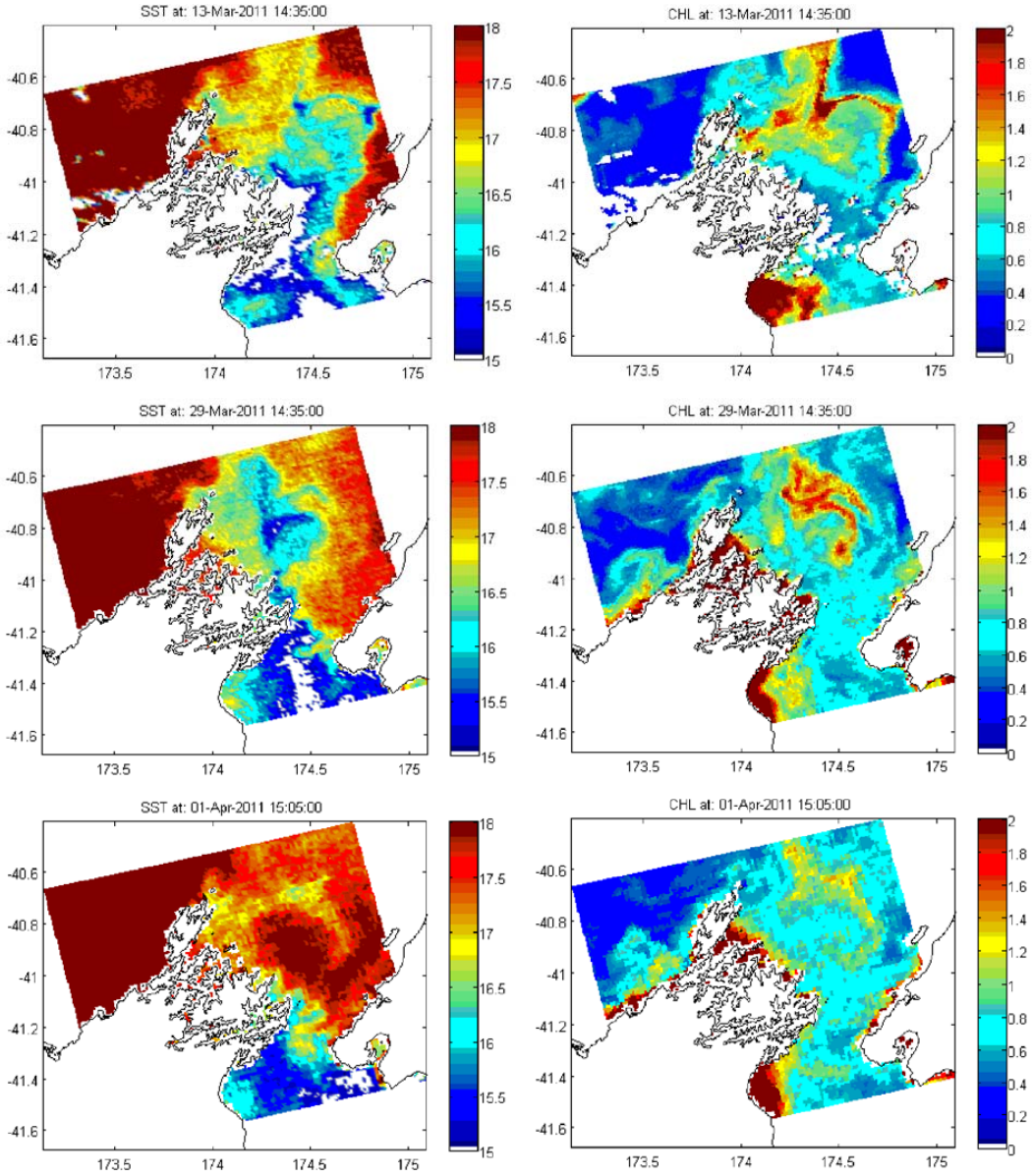


Figure 18. (continued) Satellite images of sea surface temperature and chlorophyll in the Cook Strait region, 13 March – 01 April 2011.

6. BLOOM DISPERSION MODELLING

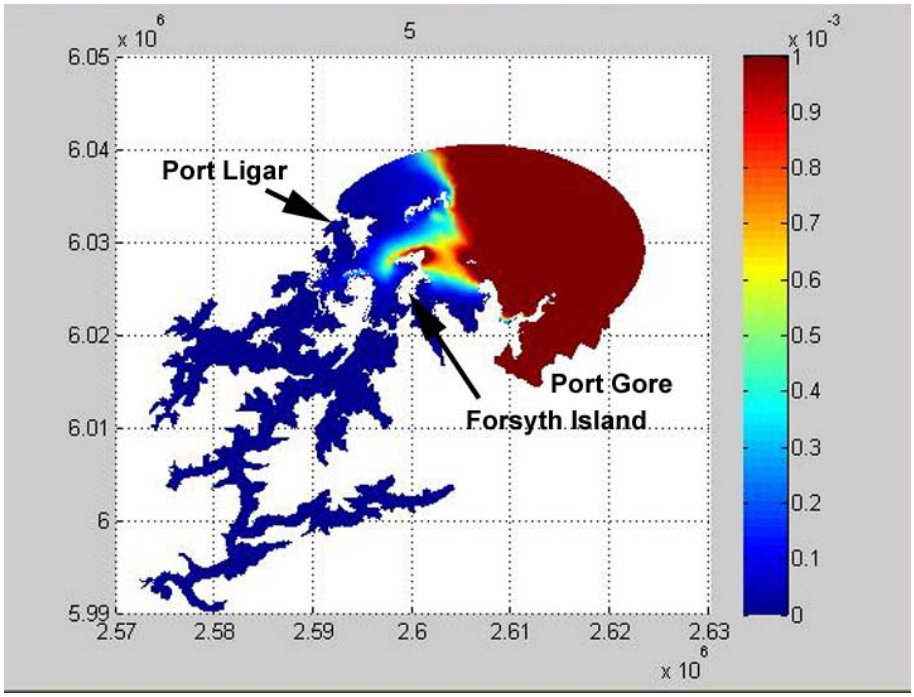


Figure 19. Particle tracking simulation with an origin in Port Gore using the 'SELFE' model run over six days.

The recent fitting of a hydrodynamic model (The SELFE model; Zhang and Baptista 2008) to the Marlborough Sounds has enabled accurate predictions of water movements in response to changes in weather conditions and offshore currents. The model was used to track the dispersion of particles introduced into Port Gore over a six day period (Figure 19). Although most particles dispersed eastward into Cook Strait the model predicted that westward movement was also possible with eddies introducing particles into the entrance to Pelorus Sound around the western side of Forsyth Island. During the March-May bloom a few cells of *A. catenella* were observed in Port Gore. These modelling results show that it is theoretically possible that cells could disperse westward from there into the major mussel farming regions of Pelorus Sound.

7. DISCUSSION AND CONCLUSIONS

At least in the medium term, the STX producing dinoflagellate *Alexandrium catenella* appears to have become established in the Queen Charlotte Sound ecosystem. Judging from experience with this species in other areas such as the Bay of Plenty, future blooms of this species in this region can be expected.

From the prevalence of resting cysts in Opua Bay, Tory Channel, it is likely that this location will be the origin of future blooms from which they may spread to other areas. It is possible that natural processes could transport cells to the mussel farming areas of Port Underwood and Pelorus Sound. A similar situation where blooms of *Alexandrium catenella* are initiated by cyst germination in one location and spread to other areas of a fjord system exists in Puget Sound, Washington USA (Cox et al. 2008).

The Queen Charlotte Sound bloom is the first occasion on which the new chemical testing programme was used to manage a real STX contamination event in New Zealand. The method performed well, and although the development of toxicity in the shellfish was sudden and occurred simultaneously with the appearance of *A. catenella* in the plankton, it was detected before levels exceeded the action threshold of 0.8 mg/kg STX equivalents as determined by the full confirmation test. Therefore, despite the rapid onset of the bloom (possibly due to mass cyst germination), there was no possibility of contaminated products entering the market chain.

The dinoflagellate bloom itself went through its development, climax and decline phases in about 40 days, although shellfish harvesting closures of up to 97 days were necessary due to the lengthy toxin clearance phase. Anything that can reduce the closure duration during this phase would help reduce the damage caused by such a bloom.

The chemical testing screen has excellent sensitivity for the detection of STXs, but for the reasons explained in the introduction, will always provide an overestimation of the real level of toxicity. The method was introduced in the context of a 20 year history of the rarity of PSP-toxins in the Marlborough Sounds so that the vast majority of samples were negative. This situation may now have changed and because the confirmation test is complex and expensive it can only be used sparingly. This creates something of a problem for reopening decisions during the low level tail of at the end of the depuration period, when screen STX equivalents (because of the high sensitivity) may hover above the permissible level for a long time. The data gathered during the bloom on the confirm/screen ratios provides a good amount of real world information to judge the optimal time to begin confirmation testing.

If more widespread closures become necessary in the future, due to expansion in the range of the dinoflagellate throughout the sounds, the need to streamline opening procedures could become a pressing issue. During the 2011 event, once the bloom had crashed there was no evidence that it could become re-established and cause renewed contamination. The toxin depuration data suggests that in the late depuration phase, as long as screen test results are below 0.8 mg/kg STX equivalents, and are remaining constant or continuing a downward

trend, there is a good amount of certainty that the real toxicity will be below 0.4 mg/kg STX equivalents, and additional confirmation should not be necessary.

8. ACKNOWLEDGEMENTS

Thanks to the Marlborough Shellfish Quality Programme (MSQP) and the Marlborough District Health Board for the use of the phytoplankton monitoring and shellfish toxicity data used in this report. Thanks also to NZ King Salmon Co. Ltd. for the use of temperature data from Te Pangu Bay, Bruce Hearn of Apex Marine for carrying out plankton and shellfish sampling at Tio Point and the various North Island biotoxin cost recovery centers for the use of their historical data on *Alexandrium catenella* distribution. The water column and cyst sampling and analyses, qPCR assays, acquisition of remote sensing data and some phytoplankton and toxin profile analyses were carried out under Cawthron's MSI funded Seafood Safety Programme (MSI contract: CAWX0703).

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