ICES Journal of Marine Science, 58: 398–403. 2001
doi:10.1006/jmsc.2000.1023, available online at http://www.idealibrary.com on IDEAL®

Short communication

Harmful algae and mariculture in New Zealand

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Rhodes, L. L., Mackenzie, A. L., Kaspar, H. F., Todd, K. E. 2001. Harmful algae and mariculture in New Zealand. – ICES Journal of Marine Science, 58: 398–403.

Harmful algal blooms and their impacts on the Greenshell™ mussel industry in New Zealand over the last decade are reviewed. The response of the regulatory authorities, seafood industry, and scientists to the first significant toxic *Gymnodinium* blooms in the summer of 1992/1993 has resulted in a well-organized interest group including scientists, commercial interests, and public health regulators. Nearly all known toxic species occur in New Zealand and unique and internationally accredited microalgal monitoring programmes have been developed. New methods, such as DNA probes, have been integrated into the system for rapid identification of species that are difficult to differentiate morphologically. Monitoring is carried out weekly, with results being dispatched within 24 h of sample receipt to enable risk assessments of toxicity by shellfish harvesters. The introduction of this system has saved the shellfish industry money and has reduced the amount of contaminated product being harvested and then rejected. All the main marine biotoxins are monitored, including paralytic, neurotoxic, diarrhetic, and amnesic shellfish toxins, and also compounds such as yessotoxin, pectenotoxin, and gymnodimine. Blooms that could affect farmed finfish or wild marine biota are also reported. Harmful algal monitoring is constantly reviewed in the light of new research and incorporates local knowledge of oceanographic and climatic conditions.

Key words: DNA probes, HABs, marine biotoxins, phytoplankton monitoring, toxic microalgae.

Received 16 October 1999; accepted 15 March 2000.

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Introduction

New Zealand's Greenshell™ mussel (*Perna canaliculus*) industry (value approximately US$62 million exported to 55 countries; Mussel Industry Council records) is acutely aware of the potential damage that products contaminated by marine biotoxins pose to its markets and to the safety of consumers. The shellfish industry funds, by levies of marine farm licence holders, weekly phytoplankton monitoring at 30 commercial harvesting sites. Microalgal biotoxin monitoring is undertaken at approximately 80 sites throughout the country, overseen by central government. Monitoring is also carried out by the Ministry of Health to assess the risk of biotoxins in shellfish to recreational shellfish gatherers, and these results are available to commercial harvesters (Figure 1).

The phytoplankton monitoring programme is the first tier of a graded response, and provides a cost-effective means of determining the potential for harmful algal bloom (HAB) development. By this means, harvesting of contaminated products can be avoided. Both this and adjustments to the flesh-testing requirements have resulted in economic benefits to the shellfish industry (Marlborough Shellfish Quality Programme, pers. comm.). The analyses of seawater samples for microalgae are carried out at the Cawthron Institute, and the laboratory has been awarded International Accreditation New Zealand (IANZ), which is covered under ISO-IEC Guide 25, the recognized international

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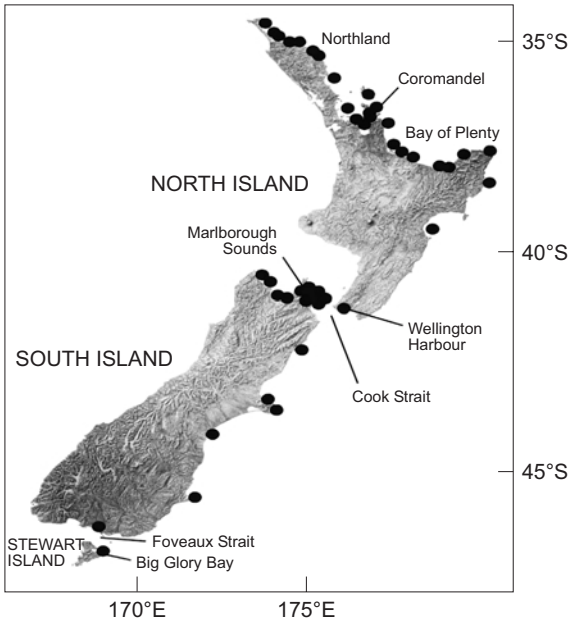

Figure 1. Map of New Zealand showing sites referred to in text (●=pytoplankton monitoring sites, 1999).

laboratory standard. This accreditation is believed to be unique.

There has been debate recently as to whether marine farming practices cause increases in either blooms dominated by toxic species or the expression of toxicity in potentially toxic species. Mariculture in New Zealand is based mostly on filter-feeding molluscs, whereas sea-cage finfish farms, which result in the introduction of "new" nutrients to the environment, are few. Although mussel farms do cause changes in the dynamics of nutrient remineralization processes within the water column and sediments, the probable net result is the removal of nutrients from the ecosystem. To date, there is no evidence that the considerable expansion in mussel farming in the Marlborough Sounds (the major shellfish growing area) over the last decade has resulted in any increased incidence of HABs.

The shellfish industry is expanding rapidly, with a great demand on water space. Over 100 applications for new marine farm sites, which could cover thousands of hectares, have recently been submitted to regional authorities for assessment. Environmental management reflects the principle of sustainability of natural resources, and mariculture is subject to New Zealand's Resource Management Act 1991 and its amendments. The HAB data collected from key farming sites will be invaluable in evaluating the impact of this activity on the environment.

The Greenshell mussel growers consider unpolluted waters as a major asset. For the protection of this asset, the Mussel Industry Council has established an environmental code of practice and supports ongoing scientific research on environmental aspects of the industry.

HABs in the past decade

Prior to 1993, monitoring of HABs amounted to seasonal analysis of seawater for finfish farmers. Regular sampling was instigated after a naturally occurring bloom of the raphidophyte *Heterosigma akashiwo* (Hada) Hada caused massive kills of caged salmon in Big Glory Bay, Stewart Island, in the summer of 1989 (Mackenzie, 1991).

Seasonal blooms in New Zealand commonly start with diatoms in early spring, followed by dinoflagellates in summer, with further late diatom blooms in autumn. In the spring of 1992, El Niño climate conditions resulted in unusually cold sea temperatures off the northeast coast and these were linked to major anomalous bloom events (Rhodes *et al*., 1993). *Mesodinium rubrum* (Lohmann) Hamburger et Buddenbrook and *Noctiluca scintillans* (Macartney) Ehrenberg were the early spring dominants, and caused problems for mussel growers because of the unappealing blood red gut contents of shellfish feeding on them. These blooms were followed by the raphidophyte *Fibrocapsa japonica* Toriumi et Takano (max. 2.4×10^5 cells l^{-1}), which covered hundreds of square kilometers of coastal waters. The ejection of trichocysts by *F. japonica* cells and subsequent entanglement of shellfish larvae in the mucus threads should be considered as a potential threat to larval recruitment and the supply of wild spat.

New Zealand has experienced several El Niño events (1992, 1993, 1997, 1998), and all have seen massive *Mesodinium*/*Noctiluca* blooms successively giving way to raphidophyte and dinoflagellate blooms, the latter commonly dominated by *Gymnodinium* species. An oceanic intrusion brought the neurotoxic bloom of *G. mikimotoi* Miyake et Kominami *ex* Oda inshore in December 1992. This bloom was responsible for the paralysis of cats fed contaminated shellfish viscera, and caused some respiratory distress in beach visitors (Jasperse, 1993; Table 1). These events triggered the biotoxin and phytoplankton monitoring programmes currently implemented.

A novel bioactive compound, gymnodimine (Seki *et al*., 1995), was isolated from Foveaux Strait oysters (*Ostrea chilensis* Philippi) following a bloom of *Gymnodinium selliforme* sp. nov. (Haywood *et al*., 2000) in Southland in 1994, and also from cultures of that microalga (Mackenzie *et al*., 1996). The blooms were linked to mass mortalities of surf clams, and were carried northwards along the South Island's east coastline with the prevailing ocean currents. Mussel harvesters in the Marlborough Sounds were warned of the event, initiated hundreds of kilometers to the south, and were able to harvest in advance of the onset. From

Table 1. Potential biotoxin producers monitored in New Zealand waters (PSP, NSP, DSP, ASP: paralytic, neurotoxic, diarrhetic, and amnesic shellfish posioning, respectively; after Hallegraeff *et al.*, 1995).

Microalga	Toxin	Human health implication
Alexandrium catenella, *A. minutum* complex, *A. ostenfeldii*, *A. tamarense*	Saxitoxins; gonyautoxins	PSP
Chattonella antiqua, *C. marina*	Breve-like	NSP
Coolia monotis	?	Unknown
Dinophysis acuta, *D. acuminata*	Okadaic acid; dinophysis toxin 2 (DTX2); pectenotoxin 2	DSP
Fibrocapsa japonica	Ichthyotoxic (?)	None
G. mikimotoi complex	Breve-like	NSP, respiratory distress
Gyrodinium galatheanum	Breve-like	NSP
Heterosigma akashiwo	Ichthyotoxic	Ass. with peppery taste
Ostreopsis siamensis	?	Uncertain
Prorocentrum lima	Okadaic acid; DTX1,4; diol esters	DSP
Protoceratium reticulatum	Yessotoxin	Uncertain
Pseudo-nitzschia australis, P. delicatissima, *P. fraudulenta*, *P. multiseries*, *P. pseudodelicatissima*, *P. pungens*, *P. turgidula*	Domoic acid	ASP

epidemiological evidence, gymnodimine is not regarded as a human health hazard and, consequently, is not regulated in the biotoxin monitoring programme. However, it does have the potential to cause false positives in mouse bioassay screen tests for neurotoxic (NSP) and diarrhetic shellfish poisons (DSP), which are based on an acetone extraction. The diethyl-ether extraction procedure (the APHA international standard method for brevetoxin detection), which is carried out when positives are recorded in the NSP/DSP screen test, does not detect gymnodimine, and this has now largely eliminated the problem (Trusewich *et al.*, 1996).

In 1998 a bloom of *Gymnodinium brevisulcatum* Chang (1999) associated with a widespread upwelling event occurred along the lower eastern North Island coast as far south as Wellington Harbour. The bloom caused mass mortalities of marine fauna and respiratory distress in human beach visitors. Toxic microalgae cells entered a major research hatchery through the seawater intake, killing batch cultures of feed microalgae, shellfish larvae, brine shrimps, and finfish. Mussels in the harbour were not killed, but rock lobsters refused to eat them (Tong, 1998). The toxin has not yet been characterized.

Blooms of *Alexandrium catenella* (Whedon et Kofoid) Balech pose a different threat to marine farmers, that of paralytic shellfish poisoning (PSP; Table 1). *Alexandrium catenella* can bloom year round, but often appears after late summer storms in the Bay of Plenty, possibly due to the disturbance of sediments which trigger the germination of cyst beds. This toxic species can be difficult to differentiate from the non-toxic *A. fraterculus* (Balech) Balech under the light microscope. *Alexandrium fraterculus* is common in the Coromandel commercial shellfish growing areas, and species designation is critical. DNA probe methods are being tested and show promise, but, currently, cell-staining techniques, which require a greater level of expertise to implement and interpret, are in use.

Regulatory constraints on mariculture

The New Zealand Marine Biotoxin Monitoring Programme was implemented in November 1993 and until October 1996 was managed by the New Zealand Marine Biotoxin Management Board. It comprises representatives from the Ministry of Health (MoH), Ministry of Agriculture and Fisheries (MAF), and the New Zealand Fishing Industry Board. A phytoplankton monitoring programme was also set up by MAF in September 1993 to complement the biotoxin programme and to provide some validation of positive biotoxin results.

In 1996, two separate programmes were established, commercial harvesting managed by MAF and recreational gathering by the MoH. The shellfish industry continues to fund the testing of shellfish flesh for biotoxins and toxic microalgal monitoring for biotoxin risk assessments. However, a review carried out by the MoH in 1996 (Wilson and Sim, 1996) determined that the human health risk posed by marine biotoxins was low compared with some other risks to public health. A reduction in the scope of the MoH surveillance programme ensued, with a focus on areas with a known history of contamination problems, and more reliance on phytoplankton density data. Another review is currently being carried out by the MoH, based on six years of systematically accumulated shellfish biotoxin and toxic microalgal data. The objectives are to assess the risk to the public of consumption of non-commercial

shellfish, and to recommend more cost-effective management and mitigation options for the future.

The need for sustainability of the marine coastal resource is spelled out in New Zealand's Coastal Policy Statement (NZCPS). Regional councils are responsible for the coastal plans required under the Resource Management Act 1991. These plans must be consistent with the NZCPS and regional councils approve marine farming leases in the light of this legislation. The Biosecurity Act 1993 was invoked by MAF in 1993. Truckloads of seaweed bearing mussel spat are collected in the far north of New Zealand after the weed has been beached during storms and are transported south to the on-growing areas. A temporary freeze on spat movement was applied when it was feared that toxic microalgae cysts could be introduced into the Marlborough Sounds by this practice (Rhodes *et al*., 1994). The ban was lifted once monitoring procedures were in place.

Phytoplankton monitoring

Phytoplankton monitoring of coastal waters is carried out in conjunction with shellfish flesh testing, and allows those responsible for the harvesting of shellfish to make a rapid risk assessment of biotoxicity. Bivalves can be left in the water to depurate if biotoxin-producing organisms are observed, with consequent reductions in financial losses following product recall.

New Zealand requires a comprehensive monitoring programme, because most known toxic microalgae are present in its waters (Table 1). Site selection depends on a variety of factors, including tidal flows, local currents, and residence time. Phytoplankton sampling is carried out on the incoming tide in bays and harbours (and upstream of marine farms), the rationale being that outgoing water will be filtered during shellfish feeding. Open ocean sites can usually be more widely spaced, as major currents carry water bodies hundreds of kilometers along the coastline. Testing of the shellfish for biotoxins is carried out on the west coast surf beaches, because of access difficulties and the danger for samplers, and of the amount of particulate matter in the water that makes sample examination difficult.

The Marlborough Sounds system is rarely nutrient limited and supports a large phytoplankton biomass. Local rivers deliver significant inputs of nitrates, largely originating from agricultural activities, into the inner and mid-Sounds following heavy rain. Upwelling in Cook Strait results in high concentrations of inorganic nutrients, which affect the outer Sounds immediately and move gradually into the mid-Sounds with tidal inflow. Local currents require that microalgal monitoring is intensive, and secondary sampling sites are designated for activation following positive biotoxin tests so that the geographic extent of closures can be minimized.

Monitoring is carried out weekly and analysis of the data collected indicates that the likelihood of undetected blooms developing, causing toxin contamination and dissipating within one week, without the presence of target cells being detected, is minimal (Todd, 1997). Incursions of oceanic waters at more open coastal sites could cause unexpected contamination events, but to date such blooms have been detected by the MoH programme and have not adversely affected growers.

Samples are collected by water bottle at 3-m depth intervals in the water column, or by hose sampler to a depth of 15 m. Two 100-ml samples are collected at each site (or depth); one is treated with Lugol's iodine fixative and the other is not fixed to assist in identifications of fragile, live cells whose morphological features can be distorted by preservation. Samples are couriered to the laboratory, where 10-ml preserved aliquots are settled in glass-bottomed chambers for 4 h, and cells identified and counted. Results are communicated to harvesters for risk assessment within 24 h of receipt. If critical levels are reached (pre-determined cell numbers per litre), the appropriate response can be taken. Critical levels were validated in an experiment funded by the government and NZ Fishing Industry Board (Todd, 1997), and are reconsidered in the light of new research data and monitoring.

DNA probe methods

Blooms of the toxic diatom *Pseudo-nitzschia* H. Peragallo are common around the coastline throughout the year (Table 1). However, several species are non-toxic or produce extremely low concentrations of toxin per cell (Rhodes *et al*., 1998). Previously, reporting of *Pseudo-nitzschia* blooms without species designation led to bivalves being unnecessarily left in the water until flesh test results (HPLC-UV detection of domoic acid) became available, usually several days after sampling. Species identification is difficult under the light microscope, but whole cell DNA probes, developed at Monterey Bay Aquarium Research Institute, USA (Miller and Scholin, 1996; Scholin *et al*., 1997), have been successfully merged into toxic algal monitoring programmes and are available on request. The use of probes means that notification of the potential toxicity of species present and their composition in the bloom can be provided within hours, leading to a marked reduction in the number of unnecessary closures (Rhodes *et al*., 1998).

Domoic acid, which causes amnesic shellfish poisoning (ASP), is produced as *Pseudo-nitzschia* blooms enter stationary phase. The toxin can remain bound to bloom debris after a bloom collapses, and the risk for shellfish harvesters exists for two weeks after whole cell DNA probe results have indicated a toxic bloom, even though actual cell numbers have dropped. The sandwich

hybridization assay (SHA) is an automated species-specific DNA probe for detecting *Pseudo-nitzschia* ribosomal RNA in seawater samples (Scholin *et al.*, 1996). Results are available within an hour of sample receipt, and the testing of this method has already led to the lifting of voluntary closures by harvesters when blooms have proved to be non-toxic. The SHA enables rRNA estimations in particulate matter when the ASP risk continues, but whole cells are no longer observable under the light microscope, by determining whether the bloom was of a potentially toxic species. The SHA system is still being tested, but will be incorporated into the monitoring programme once it has been fully tested.

Data on biotoxins, phytoplankton, environmental parameters generated from phytoplankton monitoring, and shellfish flesh testing results are stored in Foodnet, a relational database owned by the MoH. Analysis of these data may allow the detection of correlations, and the definition of benchmarks representing minimal anthropogenic influence, against which future trends can be measured.

Novel biotoxin producers

The assay for screening neurotoxins and diarrhetic toxins is based on acetone extraction but, unfortunately, tests positive for bioactivities that do not pose a human health hazard. Only about 20% of positives are due to either NSP or DSP (Truman and Stirling, 1999). For example, *Dinophysis acuta* Ehrenberg blooms annually at Wedge Point, Marlborough Sounds, and high lipotoxin concentrations in blue (*Mytilus galloprovincialis*) and Greenshell mussels as detected by mouse bioassay have not always correlated with okadaic acid (OA) as determined by DSP ELISA (Table 1). HPLC analyses led to the discovery of pectenotoxin 2 (PTX2; 23 pg cell^{-1}) in wild *D. acuta* cells. Higher concentrations of OA have been found in blue than in Greenshell mussels, and the conversion product DTX3 (7-*O*-acyl OA) has been found in Greenshell mussels (T. Suzuki, personal communication).

Investigation of anomalies between concentrations of lipotoxins detected by mouse bioassay and concentrations of OA determined by HPLC also led to the discovery of yessotoxin (YTX) in mussels. *Protoceratium reticulatum* (Claparède et Lachmann) Bütschli, a common species that co-occurs with *D. acuta* at Wedge Point, has now been shown to produce YTX (Satake *et al.*, 1997; Table 1). This compound has since been found in shellfish in other areas and is clearly responsible for many past "false positives", when no known causative microalgae were present.

In conclusion, the data collected over the past six years on both marine biotoxins and the microalgal producers will enable impact assessments of the rapidly growing Greenshell mussel industry in New Zealand in the future.

Acknowledgements

The valuable contributions of Dr Toshiyuki Suzuki, Tohoku National Fisheries Research Institute, Shiogama, Japan, Janet Adamson and Allison Haywood, Cawthron Institute, and Dr Chris Scholin, Monterey Bay Aquarium Research Institute, USA, are acknowledged. The work was supported by the Foundation for Research, Science and Technology contracts (CAW601; FIB501). Symposium attendance was made possible by a grant from the Ministry of Research, Science and Technology.

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