New Zealand Journal of Marine and Freshwater Research, 1992, Vol. 26: 371–378
0028–8330/2603&4–0371

Effects of the toxic dinoflagellate *Alexandrium tamarense* on the greenshell mussel *Perna canaliculus*

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Abstract Oxygen consumption, grazing rate, and byssus production of the greenshell mussel *Perna canaliculus* were measured following exposure to bloom concentrations (10^6 cells l^{-1}) of a toxic clone of the dinoflagellate *Alexandrium tamarense* (GT429), using a non-toxic clone of the same species (PLY173) as control. Following feeding for 1 h on GT429, mussels (dry weight 54–127 mg) showed significantly increased oxygen uptake at 15°C (0.08 µl O_2 (mg dry tissue weight)$^{-1}$) compared with control mussels. However, following 24 h recovery, oxygen uptake was similar to initial values in all experimental conditions. The grazing rate of *P. canaliculus* fed upon toxic clone GT429 was 4.58 ± 0.76 cells mg^{-1} h^{-1} compared with 1.99 ± 0.47 cells mg^{-1} h^{-1} for PLY173. On exposure to *A. tamarense*, mussels maintained normal opening behaviour and there was no change in the byssus production over 24 h. It was concluded that *P. canaliculus* showed no dramatic physiological effects following short-term feeding on the toxic strain of *A. tamarense*. During 2 weeks' exposure to twice daily bloom concentrations of GT429, there was no mussel mortality. Toxicity in the tissues was 1295 µg STX equivalent per 100 g tissue, levels at which these mussels would be unsuitable for human or animal consumption.

M92013
Received 25 February 1992; accepted 21 October 1992

Keywords *Alexandrium tamarense*; *Perna canaliculus*; red tide; oxygen uptake; byssus production; mussel ecophysiology; paralytic shellfish poisoning

INTRODUCTION

Toxic algal blooms occur in many parts of the world where they are often a common seasonal occurrence (Shumway 1990). The toxic or noxious effects of algal blooms are responsible for mass mortalities of shellfish—either as a result of direct toxicity, anoxia, or effects on the gills of filter-feeding organisms. If contaminated shellfish are consumed by humans, they may induce various forms of shellfish poisoning, including paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP). Because of the disastrous effects of toxic blooms on public health and the economics of aquaculture ventures, especially mussel farms, there have been attempts to understand the factors thought to enhance blooms and understand the sources and mode of action of the associated toxins.

Paralytic shellfish toxins are produced by certain members of dinoflagellate genera such as *Alexandrium*, *Gymnodinium*, and *Pyrodinium* which produce several tetrapurine neurotoxic analogues among the various species (Shimizu & Yoshioka 1981; Sullivan et al. 1983; Kodama & Ogata 1988; Sullivan 1988; Anderson et al. 1990). There is no historic evidence of PSP in New Zealand (Baldwin 1987; Mackenzie 1990); however, Cembella et al. (1987) have confirmed the presence of paralytic shellfish toxins (GTX_{1+4}, GTX_{2+3} and STX) in a dinoflagellate isolate from Whangarei. Further, there is concern about possible introductions via cysts in the ballast water of visiting ships (Hallegraeff & Bolch 1991).

Alexandrium tamarense is the organism responsible for PSP in many parts of the world and its effects are the best documented of any of the toxic species. The effects on mussel species including the

blue mussel *Mytilus edulis*, the horse mussel *Modiolus modiolus*, and the brown mussel *Perna perna* are well known (Ayres & Cullum 1978; Reyes-Vasques et al. 1979; Fraga & Sanchez 1985; Gainey & Shumway 1988). *A. tamarense* influences the physiology of certain bivalves by affecting heart beat, filtration rates, and feeding ability (Shumway et al. 1985; Shumway & Cucci 1987; Gainey & Shumway 1988). *A. tamarense* has also been shown to reduce byssus production in *M. edulis* and *Geukensia demissa* (Shumway et al. 1987).

The present study was undertaken to investigate the potential effects of the toxic dinoflagellate *A. tamarense* on the New Zealand greenshell mussel. Although blooms of algae responsible for paralytic shellfish poisoning have not been recorded in New Zealand, a toxic strain of an "*Alexandrium*-like" dinoflagellate has been isolated (Cembella et al. 1987). It is not known if *P. canaliculus* has previously been exposed to this or similar toxic dinoflagellates. Experiments were designed to estimate the effects of a toxic strain of *A. tamarense* (GT429) on grazing rate, oxygen uptake, byssus production, and accumulation of PSP toxins. A non-toxic strain of *A. tamarense* (PLY173) was used as the control.

MATERIALS AND METHODS

P. canaliculus collected from the Marlborough Sounds (41°00′S, 174°00′E) during winter was transported by air to Boothbay Harbor, Maine, United States in June 1989. They were maintained in quarantine conditions at 15 ± 2°C in running sea water that was filtered through a sand filter to remove all particles > 20 µm. No supplementary food was given over the storage period. Mussels were allowed to adjust to the new natural photoperiod and constant laboratory conditions for 2 weeks before any experimentation. Samples of *A. tamarense* (Clones GT429 and PLY173) were provided by the Provasolli-Guillard Culture Center for Marine Phytoplankton located at the Bigelow Laboratory for Ocean Sciences. They were grown in laboratory culture in f/2 medium at 15°C with a L:D = 14:10 photoperiod. The phytoplankters were used in experiments during the logarithmic part of their growth cycle. Cultures were diluted to bloom concentrations of c. 10^6 cells l^{-1} with 0.45 µm millipore-filtered sea water.

Oxygen consumption

Oxygen uptake of *P. canaliculus* was measured using a Gilson differential respirometer. As there were no published records of $\dot{V}O_2$ in this species, a preliminary experiment was undertaken to provide baseline values and to assess the effects of body size and temperature change on aquatic oxygen uptake. A group of 14 mussels (length 21.6–31.7 mm) was used in the experiment which measured oxygen uptake of individual mussels following acute temperature change in the sequence 10, 5, 15, and 20°C. The 50-ml chambers containing the mussels were shaken at slow speed to facilitate gas exchange, allowing 1 h equilibration at the start of the experiment and following each temperature change.

For experiments investigating the effects of *A. tamarense* on the oxygen uptake of *P. canaliculus*, mussels were individually numbered using plastic dots at least 4 days before experimentation. Pre-feeding levels of oxygen uptake were measured in 14 individuals (length 28.8–34.8 mm) at 15°C following 1 h equilibration, taking measurements every 5 min for 60 min. Two groups of 5 mussels were transferred individually into vessels containing 350 ml of feeding mixture, either GT429 (1.7×10^3 cells ml^{-1}) or PLY173 (2.57×10^3 cells ml^{-1}). The remaining 4 control mussels were placed into 0.45 µm filtered sea water. After 1 h exposure, a 10 ml sample of the dinoflagellate culture used for the feeding experiment was removed for particle counting. Cell counts were made using a microscope with a 10× objective and Spiers-Levy hemocytometer following the procedure outlined by Guillard (1973). Mussels were then returned to the respirometer flasks containing 0.45 µm-filtered sea water. After the 1 h equilibration period, oxygen uptake was measured for a further 1 h. Mussels were returned to the aquarium overnight at 15°C and the oxygen consumption was reassessed 24 h after the initial exposure to the dinoflagellate cultures. At the end of the experiments, dry tissue weight and dry weight condition index (CI) = dry tissue weight/dry shell weight (Crosby & Gale 1990) were calculated for all individuals.

Byssus production

Three groups of 20 mussels (length 26–65 mm) were individually marked with small numbered plastic dots for the byssus experiments. Previously produced byssus threads were cut with scissors and animals were maintained in aerial, humid conditions for 12 h to encourage byssus production (van Winkle 1970). After measurement of byssus production, mussels were maintained for 7 days before exposure to GT429, PLY173, or control (filtered sea water). After another 7 days' recovery, post-exposure levels of byssus

production were measured in the same way. From each separated group of 20 mussels, 15 were chosen at random for each experiment. Pre-feeding levels of byssus production were measured at 20°C with mussels held individually in aerated shallow containers with 200 ml of 0.45 µm filtered sea water. Shell opening behaviour of mussels was observed at time intervals of 0.5, 1, 2, 6, and 24 h. The number of byssus threads produced over the 24 h exposure time was noted, together with the presence of any faeces or pseudofaeces. After the initial estimates of byssus production, mussels were maintained for 7 d in the aquaria before exposure to dinoflagellates or control conditions using filtered sea water. The cell concentration of *A. tamarense* was 2.0×10^3 cells ml^{-1}. Following 1 h exposure, a single 10 ml sample was removed for estimation of grazing rates. A further sample was taken after 24 h to monitor feeding activity. Mussels were returned to the storage aquaria, and following a further 7-day recovery period, shell opening, and faecal and byssus production were compared with the original values. At the end of the experiments the dry tissue weights and CI were calculated for each mussel. As the starting times of the experiments were staggered, the total duration of the experiments and control was 30 days. Samples of at least 13 mussels were taken at intervals from the quarantine animals for CI values to check for any possible changes in mussel condition over time.

Long-term exposure to GT429

A preliminary experiment was undertaken to measure the possible long-term effects of exposure to GT429. Two groups of 6 mussels (length 52–57 mm) were held individually in 350 ml of filtered sea water at laboratory temperature 15°C. The experimental group was exposed twice daily to bloom concentrations (10^3 cells ml^{-1}) of GT429. Mussels were checked daily to see if they were showing normal opening or feeding behaviour. Following 14 days' exposure, the mussel tissue from individuals within each group was combined and tested for PSP toxins using the mouse bioassay (Horowitz 1984; Sullivan 1988).

RESULTS

Figure 1 shows the regression lines describing the aquatic oxygen uptake for *P. canaliculus* as a function of dry tissue weight at various experimental temperatures. All the lines were significant at $P = 0.01$. Oxygen uptake ($\dot{V}O_2$) increased with increasing body weight with a similar weight exponent at temperatures

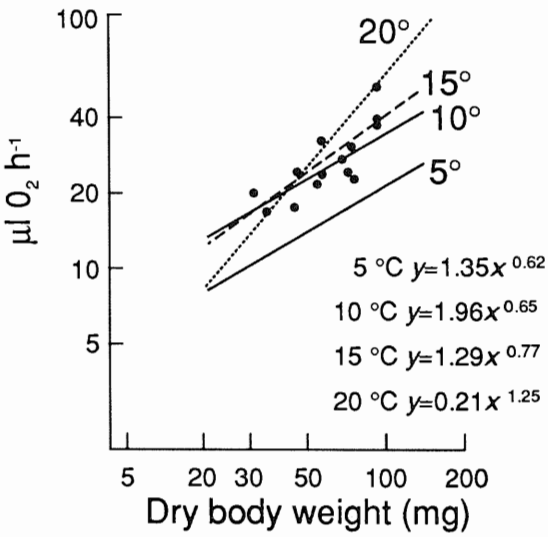

Fig. 1 Effect of size on the $\dot{V}O_2$ of *P. canaliculus* at different exposure temperatures. Also shown are the regression equations which were all significant at $P = 0.01$. For 15°C, both the regression line and the points associated with the line are shown.

of 5, 10, 15 and 20°C (analysis of covariance (ANCOVA) F constant = 1.82, d.f. 3:46, $P = 0.25$). Temperature had a significant effect on oxygen uptake (ANCOVA F constant = 18.85, d.f. 3:49, $P = 0.05$), with oxygen uptake at 5°C being significantly reduced compared with the other exposure temperatures (ANCOVA F constant = 9.13, d.f. 1:25, $P = 0.01$).

Rates of oxygen uptake of *P. canaliculus* recorded 1 h after the feeding episode were within the range recorded in the initial experiment. Oxygen uptake values were corrected to a standard dry body tissue weight of 90 mg, using the weight exponent $b = 0.77$ recorded at 15°C in the previous experiment. Table 1 shows the oxygen uptake (µl O_2 mg^{-1} h^{-1}) for mussels at the start of the experiment, following 1 h exposure to experimental cultures and following 24 h recovery. For both the controls and mussels exposed to PLY173, there was no effect of experimental manipulation or time on the oxygen uptake. Following feeding on GT429, oxygen uptake of *P. canaliculus* was elevated 0.08 µl mg^{-1} compared with pre-feeding levels (ANOVA F constant = 10.0, d.f. 2:6, $P = 0.05$). Following 24 h recovery, oxygen levels approximated the initial values. The grazing rate ($\bar{x} \pm$ SE) for *P. canaliculus* feeding on toxic GT429 was 4.58 ± 0.76 cells mg^{-1} h^{-1}, which is significantly higher than the 1.99 ± 0.47 for the non-toxic PLY173 ($P = 0.05$).

On exposure to *A. tamarense*, most mussels opened within 0.5 h (Table 2) and for long periods kept their valves open and the mantle edges slightly protruded. In all experimental conditions, the mussels in the feeding trials showed similar results to the pre-feeding trials. No adverse effects of the dinoflagellates such as the milky or mucous discharges or pseudofaeces production noted in other species (Gainey & Shumway 1988) were recorded. All mussels that had fed on GT429 produced normal faeces. Over the first hour of the byssus production experiments, individual mussels exposed to dinoflagellates consumed similar numbers of GT429 cells ($\bar{x}$ = 172.1 ± 20.6) and PLY173 ($\bar{x}$ = 170.3 ± 20.2). Mean byssus production in *P. canaliculus* (Table 3) ranged between 3.4 and 6.2 threads per individual over any 24 h exposure period. There was no significant reduction in byssus production in mussels exposed to either GT429 or PLY173 (*t*-tests). Mean (± SD) byssus production for mussels exposed to GT429 (combined for the three time intervals) was 5.9 ± 3.9 and 4.2 ± 3.0 for PLY173, and 4.8 ± 2.9 for the control. The effect of exposure time on byssus production in *P. canaliculus* is shown by the combined results from the three experiments in Fig. 2, which also illustrates the CI of independent samples. Byssus production declined linearly over time following the equation: production = 6.2–0.07 time (days), r = 0.72, P = 0.05. Although over this period the average CI of mussels was reduced, no direct relationship was evident between mean byssus production and mean CI index for mussels following the recovery period (ANCOVA).

Individuals of *P. canaliculus* exposed to bloom concentrations of GT429 twice daily were exposed to an estimated 9 × 10^6 cells over the 14-day period.

Table 1 Effects of exposure to *Alexandrium tamarense* cultures on $\dot{V}O_2$ (µl O_2 mg^{-1} h^{-1} ± SD) in *P. canaliculus*. F is the value for the ANOVA comparisons of the mean values at the different time intervals. * indicates the only experiment where values differed significantly from initial values (P = 0.05).

	Initial	1 h after feeding	24 h recovery	F
GT 429	0.43 ± 0.11	0.51 ± 0.17*	0.43 ± 0.13	2.79 (P = 0.09)
PLY 173	0.42 ± 0.14	0.40 ± 0.10	0.36 ± 0.14	0.26 (P = 0.77)
Control	0.39 ± 0.14	0.40 ± 0.05	0.34 ± 0.07	0.46 (P = 0.64)

Table 2 Opening behaviour and faecal production (Fp) after 24 h for *P. canaliculus* exposed to PLY173 and GT429. All values are percentages. Pre-expt, initial values. Expt, after 7 days; Recovery, 7 days after feeding.

	Time (h)					
	0.5	1	2	6	24	Fp
GT429						
Pre-expt	100	93	93	86	80	87
Expt	100	86	86	86	71	100
Recovery	87	100	100	86	73	100
PLY173						
Pre-expt	100	100	93	87	66	47
Expt	93	93	100	100	100	80
Recovery	93	93	100	73	67	79
Control						
Pre-expt	100	100	100	100	100	100
Expt	87	93	100	100	93	80
Recovery	80	100	100	100	73	93

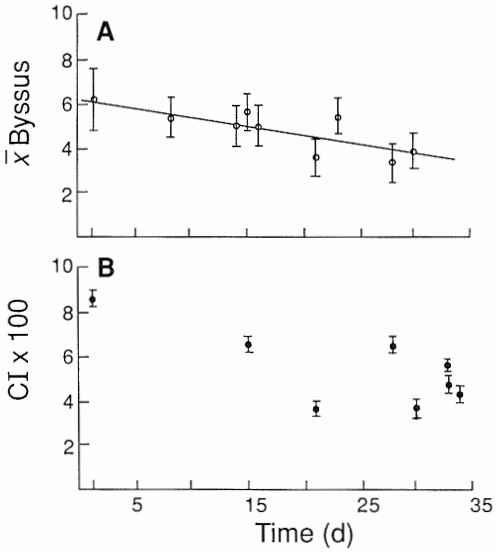

Fig. 2 Effects of quarantine time on **A**, mean byssus production ± SE over a 24-h time period; and **B**, the mean condition index (CI) ± SE of *P. canaliculus*.

This corresponded to an estimated total exposure of c. 2.7×10^4 cells mg^{-1} dry weight tissue. There was no mortality in either the experimental or control group, and mussels showed normal opening, feeding behaviour, and byssus production. The toxicity levels recorded from tissues of mussels exposed to GT429 was 1295 µg STX equivalents per 100 g wet weight tissues compared with control mussels which were < 58.0 (at the lower sensitivity limit of assay).

DISCUSSION

Several recent studies have investigated the physiological and behavioural effects of the toxic dinoflagellate *A. tamarense* on mussels. Although the responses are largely species-specific, they can also vary within a species, depending on the location where specimens were collected and their likely previous exposure to red-tide dinoflagellates (Shumway et al. 1987). Certain mussel populations that have not been exposed previously to toxic blooms may show immediate or delayed valve closure and may minimise filtration rates. Such responses were seen in *Mytilus edulis* from both Rhode Island and Spain (Gainey & Shumway 1988), thus effectively reducing exposure to dinoflagellate toxins. The lack of a similar closure response in *P. canaliculus* suggests this mussel does not respond in the short term to the potentially harmful effects of the toxic dinoflagellate *A. tamarense*.

Previous studies have shown that bivalves differ in their filtration response on exposure to toxic dinoflagellates. Several species, including some oysters and clams showed reduced filtration (Ray & Aldrich 1967; DuPuy & Sparks 1968; Shumway & Cucci 1987) whereas others, such as *M. edulis* and *Ostrea edulis* from Maine, exhibited increased filtration rates following exposure to GT429 (Shumway & Cucci 1987). *P. canaliculus* readily feeds on the toxic *A. tamarense* clone GT429 with a grazing rate similar to those calculated at similar temperatures for the mussel *M. edulis* (Cucci et al. 1985). In the two experiments described here, *P. canaliculus* was able to maintain its filtration rate upon exposure to GT429, and in this respect it is similar to the sea scallop *Placopecten magellanicus* and the Atlantic surfclam *Spisula solidissima* (Shumway & Cucci 1987).

Differences in the grazing rate between the toxic and non-toxic clones of *A. tamarense* cannot be explained by the present experiments. Shumway et al. (1990) describe three different selection mechanisms for bivalves including the ctenidia, the ciliated grooves of the labial palps, and the ciliated tracts of the gut wall. The cell size for both GT429 and PLY173 is 35 µm and it is likely that the retention of these particles in *P. canaliculus* results from selection and retention by the labial palps. This mussel did not produce pseudofaeces under the experimental conditions in the presence of *A. tamarense* and also maintained a low, variable rate of byssus production. In this respect, *P. canaliculus* differed from other mussels that have not previously been exposed to PSP-toxin producing algal blooms. *Mytilus edulis*, *Modiolus modiolus*, and *Geukensia demissa* collected from such areas showed inhibition of byssus production following exposure to GT429 (Shumway et al. 1987). These authors suggested that the reduced

Table 3 Number of byssus threads ($\bar{x}$ ± SD) produced by *P. canaliculus* during 24 h experiment. Pre-expt, 7 days before experimental manipulation; Expt, exposure to GT429, PLY173, or control; Recovery, 7 days after feeding.

	Pre-expt	Expt	Recovery
GT429	6.2 ± 4.9	5.4 ± 3.8	5.6 ± 3.1
PLY173	5.1 ± 3.5	3.6 ± 2.8	3.4 ± 2.3
Control	5.0 ± 3.3	5.5 ± 3.1	3.9 ± 2.4

byssus production may be a whole-animal physiological stress response induced by dinoflagellates. Byssus production in *P. canaliculus* was reduced over the storage period of 30 days and it seems likely that in *P. canaliculus*, the byssus production reflects the overall metabolic level of the mussel.

Many external and internal factors affect oxygen uptake in mussels (for reviews see Bayne et al. 1976; Newell 1979) including size, temperature, season, and nutritional status. *P. canaliculus* reaches a larger maximal size than other mussels; however, its weight-specific $\dot{V}O_2$ was similar to that of *M. edulis* and *M. californianus* collected in comparable seasons and exposed to similar temperatures (Bayne 1973; Bayne et al. 1976). During experimental manipulation, juvenile *P. canaliculus* exposed to non-toxic *A. tamarense* (PLY173) showed similar oxygen uptake 1 h after feeding and following 24 h recovery. In all instances, $\dot{V}O_2$ was similar to sea water controls. In contrast, oxygen uptake increased by c. 20% following 1 h exposure to toxic GT429, with the rate declining to prefeeding levels within 24 h. Relatively few studies have investigated the effects of feeding activity on the oxygen uptake of bivalves. In a study on *Mytilus edulis*, Langton (1975) recorded an elevated oxygen uptake close to the feeding time and Bayne (1976) has attributed this to the specific dynamic action (SDA) representing the metabolic cost of feeding. Bayne (1976) and Bayne & Scullard (1977) reported larger increases in oxygen uptake in *M. edulis* following feeding on *Tetraselmis suecica* than recorded here for *P. canaliculus* feeding on GT429. In another study where oxygen uptake of bivalves has been measured following exposure to toxic dinoflagellates, the results have been variable (Gainey & Shumway 1988). Species such as *Mya arenaria* showed an increase in oxygen consumption, *Spisula solidissima* showed a decline, and *M. edulis* from Maine showed no change in oxygen uptake. In contrast, *M. edulis* from Rhode Island (no prior exposure to toxic dinoflagellates), showed an increase in oxygen consumption and an increase in valve closure. The results presented here for *P. canaliculus* show increased oxygen uptake without the initial valve closure and response recorded in previous studies. The increased oxygen uptake representing the SDA was, however, not evident following 24 h recovery—suggesting no long-term respiratory effect of the toxic dinoflagellates on the mussel.

Although *P. canaliculus* had not previously been exposed to blooms of the toxic algae GT429, mortality in experimental conditions was negligible. Mussels accumulated PSP toxins at a rate similar to that recorded for other mussel species. Toxicity levels following 2 weeks' exposure were similar to the highest values previously recorded for field-collected *M. edulis* from Maine (Shumway et al. 1988). Generally, mussels accumulate PSP toxins faster than most other species of shellfish and correspondingly appear to eliminate the toxins quickly (Shumway 1990). This contrasts with quahogs, *Mercenaria mercenaria* which accumulate toxins slowly (Bricelj et al. 1991) and have behavioural mechanisms to isolate themselves from toxic blooms (Bicknell & Collins 1973; Bond & Lachance 1959). In *P. canaliculus*, twice-daily exposure to bloom conditions of GT429 resulted in high accumulation rates equivalent to those found in *M. edulis*. Bricelj et al. (1990) exposed *M. edulis* and *M. mercenaria* from Long Island, New York, to the toxic dinoflagellate *Alexandrium fundyense* and showed the amount of toxin ingested increased linearly with time, with mussels reaching maximal toxicity levels between 12 and 13 days. For *M. edulis*, high toxicity levels can be retained from 10 to 50 days depending on the initial toxicity level (Shumway 1990).

The results of the present study clearly demonstrate that *P. canaliculus* has the ability to survive, feed normally, and accumulate paralytic shellfish toxins from toxic dinoflagellates. Studies by Waite (1989) suggest that *P. canaliculus* may feed on a wide size range of potential food items, suggesting a dietary composition which might include several noxious species including red-tide organisms, brown-tide organisms, and noxious diatoms (MacKenzie 1990). Some toxic phytoplankton have been identified from New Zealand waters and are known to be associated with fish mortalities (Chang 1987a, 1987b; MacKenzie 1989; Chang et al. 1990). These include the raphidiophycean micro-flagellate *Heterosigma akashiwo* which was responsible for salmon mortality in Stewart Island during the summer of 1988/1989 (Chang 1990). Also, small losses of caged chinook salmon have resulted from blooms of another toxic dinoflagellate, *Gyrodinium aureolum* (Todd & Hickman 1990). Although such blooms have reportedly not affected shellfish beds, there is little documented information (MacKenzie 1990). Regular monitoring of shellfish for PSP or DSP toxins occurs under the New Zealand Shellfish Sanitation Programme but there is no regular field-monitoring scheme as is present in other countries (Shumway et al. 1988).

Although no documented cases of paralytic shellfish poisoning have been reported from New Zealand waters, some scientists believe that the

numbers of toxic algal species and the numbers of toxic blooms are increasing globally (Shumway 1990; Smayda 1992). In 1983, a new species of "*Alexandrium*" type dinoflagellate was isolated from a population of algae associated with fish and shellfish kills off the Northland, New Zealand coast. This isolate was subsequently shown to produce PSP toxin in culture (Cembella & Taylor 1985; Cembella et al. 1987). Toxic dinoflagellates can be inadvertently introduced when their cysts are discharged with the ballast tank sediments of bulk container ships. In Australia, for example, 6% of cargo vessels tested carried the toxic dinoflagellates *A. catenella* and *A. tamarense* in ballast sediments, estimated at up to 300 million cysts per ship (Hallegraeff & Bolch 1991). Although there are now water quarantine regulations in place to minimise the introduction of exotic species into New Zealand, the presence of these species in Australian waters provides a continuing threat to the New Zealand mussel industry. This highlights the need for regular monitoring of coastal plankton for toxic species and the need for further studies to examine their potential effect on commercial shellfish.

ACKNOWLEDGMENTS

The work was undertaken whilst I. D. Marsden was on study leave from the University of Canterbury. We thank Jan Barter for help in the maintenance of algal cultures. We appreciate the co-operation of the FDA/NMFS Marine Biotoxin Survey Contract Committee. This study was funded in part by a U.S. Food and Drug Administration contract (223-89-4064) to the Massachusetts Health Research Institute and in part by grant/cooperative agreement (NA-90-AA-H-SK030) from the National Oceanic and Atmospheric Administration awarded to the New England Fisheries Development Association. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. This is Bigelow Laboratory for Ocean Sciences contribution no. 92006.

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