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To cite this article: F. H. Chang , Y. Shimizu , B. Hay , R. Stewart , G. Mackay & R. Tasker (2000) Three recently recorded *Ostreopsis* spp. (Dinophyceae) in New Zealand: Temporal and regional distribution in the upper North Island from 1995 to 1997, *New Zealand Journal of Marine and Freshwater Research*, 34:1, 29-39, DOI: [10.1080/00288330.2000.9516913](https://doi.org/10.1080/00288330.2000.9516913)

To link to this article: <http://dx.doi.org/10.1080/00288330.2000.9516913>



Published online: 29 Mar 2010.



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Three recently recorded *Ostreopsis* spp. (Dinophyceae) in New Zealand: temporal and regional distribution in the upper North Island from 1995 to 1997

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Abstract Three species of epiphytic dinoflagellates—*Ostreopsis siamensis*, *O. lenticularis*, and *O. ovata* have recently been found on both the east and west coasts of the upper North Island, New Zealand. The morphological differences of all three

Ostreopsis spp. have been studied with both light and scanning electron microscopes. Detailed studies of the inner face of the thecal wall of *Ostreopsis siamensis* revealed two types of trichocyst pores: small, simple pores and large multipore structures. The multipore structures apparently have not been previously reported. Surveys conducted during the period from November 1995 to April 1997 showed that all three *Ostreopsis* spp. were fairly widespread in northern New Zealand, and all three species were found to occur south of 35°S latitude. Cell concentrations of *Ostreopsis* spp. in summer were substantially higher than in early spring. There was also a clear regional difference in distribution; cell concentrations of the dominant species, *O. siamensis*, were greatest in Rarawa and Tokerau on the north-east coast. Other less abundant epiphytic species recorded during the same period included *Prorocentrum lima*, *P. compressum*, and *Coolia monotis*. These species were generally more sporadic in distribution than *Ostreopsis* spp.

Keywords epiphytic dinoflagellates; *Ostreopsis siamensis*; *O. lenticularis*; *O. ovata*; *Coolia monotis*; *Prorocentrum lima*; cell abundance; temporal and regional distribution

INTRODUCTION

Since 1994, during routine screening conducted by the New Zealand Ministry of Agriculture and Fisheries Regulatory Authority, low levels of unidentified lipid-soluble toxins have constantly been detected by mouse bioassay in shellfish collected from Rangaunu Harbour on the north-east coast of New Zealand. These lipid-soluble toxins apparently are neither brevetoxin nor yessotoxin, as specific tests on the extracts did not produce positive results as expected for these toxins (New Zealand Marine Biotoxin Management Board). As a consequence oyster harvesting has been banned for considerable periods over the last few years. On February 1995 surface plankton samples collected from Rangaunu

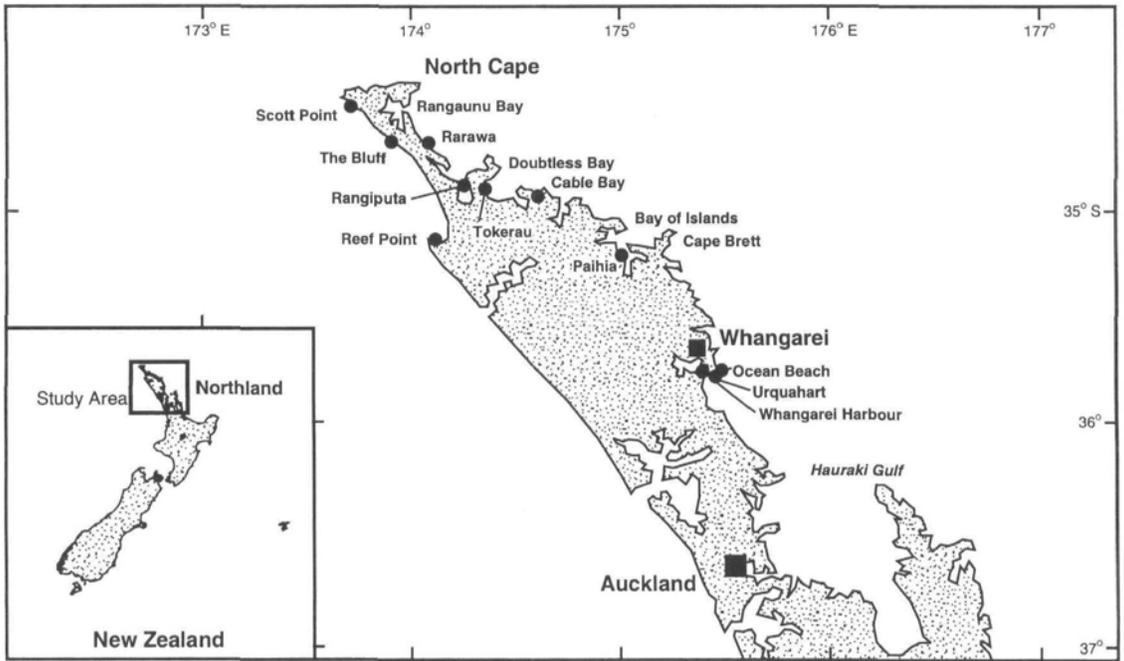


Fig. 1 Study area (inset) and sampling locations (●) on the east and west coast of the upper North Island, New Zealand.

Harbour were found to contain large numbers of *Ostreopsis* spp. and lesser numbers of a *Coolia* sp. This prompted the search for all potentially toxic epiphytic dinoflagellate species in the region.

Ostreopsis and *Coolia* are two of the five common genera (e.g., *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, *Amphidinium*) found in ciguatera-endemic, tropical regions. In the past this group of epiphytic/benthic dinoflagellates has been linked to ciguatera fish/seafood poisonings (CFP/CSP). Consumption of tropical fish and seafood contaminated by a number of these organisms, have been suggested as the cause of the CFP and CSP outbreaks overseas (Yasumoto 1971; Halstead 1973; Fukuyo 1981; Murakami et al. 1982). However, no CFP/CSP has ever been either reported or confirmed in New Zealand.

Ostreopsis and a number of other ciguatera causing organisms are known to have a world-wide distribution, and have consistently been recorded between 35°N and 34°S latitude within the tropical and subtropical regions of the Pacific (e.g., Fukuyo 1981; Bagnis et al. 1985; Gillespie et al. 1985; Holmes et al. 1988; Faust et al. 1996; Usup et al. 1997; Holmes et al. 1998), Atlantic (e.g., Carlson & Tindall 1985; Ballantine et al. 1988; Tindall et al.

1990; Morton & Faust 1997), and Indian Oceans (e.g., Quod 1994, 1995; Faust 1995). But in New Zealand very little is known about this group of epiphytic dinoflagellates, except *Prorocentrum lima* and *Coolia monotis* (Rhodes & Syhre 1995; Rhodes & Thomas 1997). This study aims to investigate how widespread these potentially toxic epiphytic dinoflagellates are in northern New Zealand, and whether there is a seasonality in abundance of these species. This study was conducted over the period from November 1995 to April 1997.

STUDY AREA

The study areas were located at the far north of New Zealand stretching from 34°30'S to 35°40'S latitude, and were made up of close inshore embayments on the east coast and open sandy beach on the west coast of the upper North Island (Fig. 1). Nearshore waters on the north-east coast are bounded at the shelf edge by the East Auckland Current, a warm high salinity subtropical current (Sharples 1997; Chang et al. 1998). Typically in summer, warm surface subtropical waters intrude into nearshore waters (Sharples 1997).

On the north-east coast, several sites in the vicinity of Rangaunu Harbour are important commercial shellfish harvesting areas. During the period from November 1995 to April 1997, seaweed samples were collected from the core areas, Rangiputa, Tokerau, Rarawa on the north-east coast, and one site from Reef Pt on the north-west coast of the upper

North Island (Table 1). In March and September/October 1996 additional samples were collected from Cable Bay, Paihia, Urquhart, Whangarei Wharf, and Ocean Beach on the north-east coast, and Scott Pt and The Bluff on the north-west coast of the North Island (Fig. 1). In April 1997 surface seawater samples were collected from Rarawa and Tokerau.

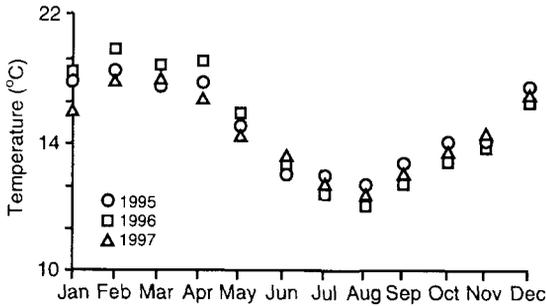


Fig. 2 Mean monthly sea surface temperatures at Ahipara Bay near Reef Pt on the west coast of the upper North Island, New Zealand, between 1995 and 1997.

Table 1 Locations on north-east coast of North Island, New Zealand, where seaweed samples were collected from November 1995 to April 1997.

Location	Site no.	Date	Temperature (°C)	Remarks
Seaweed samples				
Rangiputa	1	1 Nov 1995	15.5	—
Tokerau Beach	2	2 Nov 1995	15.4	—
Cable Bay	3	2 Nov 1995	16.0	—
Paihia/Opua	1	6 Mar 1996	22.5	southern end Pahia Beach
Cable Bay	2	4 Mar 1996	22.3	large swell
Tokerau Beach	3	4 Mar 1996	21.8	water murky
Rangiputa	4	4 Mar 1996	22.0	—
Rarawa Beach	5	5 Mar 1996	22.1	very clear blue water, sandy beach
Scott Pt	6	4 Mar 1996	21.4	very large swell (no seaweed other than kelp sample taken)
The Bluff	7	4 Mar 1996	21.0	large waves breaking on Bluff, only bull kelp on exposed rocks taken
Reef Pt	8	5 Mar 1996	22.0	—
Cable Bay	1	24 Sep 1996	—	strong easterly, and swell
Tokerau Beach	2	24 Sep 1996	15.9	water murky headland with strong easterly
Rangiputa	3	24 Sep 1996	15.5	sheltered
Rarawa Beach	4	24 Sep 1996	16.5	very strong easterly, swell
Reef Point	5	24 Sep 1996	17.1	relatively sheltered from easterly
Urquhart's Bay	1	23 Oct 1996	16.4	strong northwesterly
McKenzie Bay	2	22 Oct 1996	16.0	relatively sheltered
Ocean Beach	3	22 Oct 1996	15.3	strong northwesterly
Water samples				
Rarawa	1	3 Apr 1997	20.2	—
Tokerau	2	3 Apr 1997	20.1	—

were collected (Table 1 and Fig. 1), and where possible they were sorted and placed in separate jars. On the west coast sandy beach sites foliose seaweeds with substrates were difficult to find. Under these circumstances drifting seaweeds were collected instead.

All seaweeds and sea water collected were immediately fixed in a final concentration of 5% formaldehyde in a 500 ml plastic jar. Four batches (twice per year) of seaweed samples were collected between November 1995 and April 1997.

Seaweed samples were processed in the laboratory by shaking vigorously in a jar for a couple of minutes, and rinsing them 3 times with freshly prepared 5% formaldehyde in sea water (c. 200 ml in each rinse). The epiphytic dinoflagellates washed off the seaweeds in all rinses were pooled together, and then sieved through 100 µm nylon mesh to remove extraneous matter and left to settle overnight. The supernatant was withdrawn using a suction pump at low pressure the following day and samples reduced to a final volume of 100–200 ml.

Cell enumeration and species identification

Triplicate subsamples (up to 8 ml depending on cell concentrations) of formaldehyde-fixed cells were settled in a sedimentation chamber overnight before enumeration and identification of epiphytic dinoflagellate species using a Nikon Diaphot inverted microscope at 100× and 200×. The presence and the number of epiphytic dinoflagellates in each sample were both tabulated. Cell concentrations presented in Tables 3 and 4 are means of triplicate subsamples. Cell number of each species was expressed per gram fresh weight (FW) seaweed (cells g⁻¹ FW seaweed) (Gillespie et al. 1985).

Specimens of *Ostreopsis* spp. from several sites were also prepared for scanning electron microscopy (SEM). Cells were postfixated with 2% OsO₄ for 1 h at room temperature, rinsed with distilled water and collected on 25 mm diameter Nuclepore polycarbonate filter (2 µm pore size). Portions of this filter were dehydrated in increasing concentrations of acetone, critical-point-dried, and sputter-coated with

Table 2 Distribution of epiphytic dinoflagellates on seaweeds and in water samples collected at all sites on the upper North Island, New Zealand.

Locations	<i>Ostreopsis siamensis</i>	<i>O. lenticularis</i>	<i>O. ovata</i>	<i>Prorocentrum lima</i>	<i>Coolia monotis</i>
(1) Seaweed samples					
Nov 1995					
Rangiputa	+	+	+	-	-
Tokerau	+	-	-	-	+
Cable Bay	+	+	-	+	-
Mar 1996					
Rangiputa	+	+	+	+	+
Tokerau	+	+	+	-	+
Cable Bay	+	+	+	-	-
Rarawa	+	+	+	-	-
Reef Point	+	+	+	-	-
Sep 1996					
Rangiputa	+	-	-	-	-
Rarawa	+	+	-	-	-
Tokerau	-	-	-	-	-
Reef Point	-	-	-	-	-
Oct 1996					
Urquhart's Bay	+	-	-	-	-
McKenzie Bay	-	-	-	-	-
Ocean Beach	-	-	-	-	-
(2) Water samples					
Apr 1997					
Rarawa	+	+	-	-	-
Tokerau	+	+	+	-	+

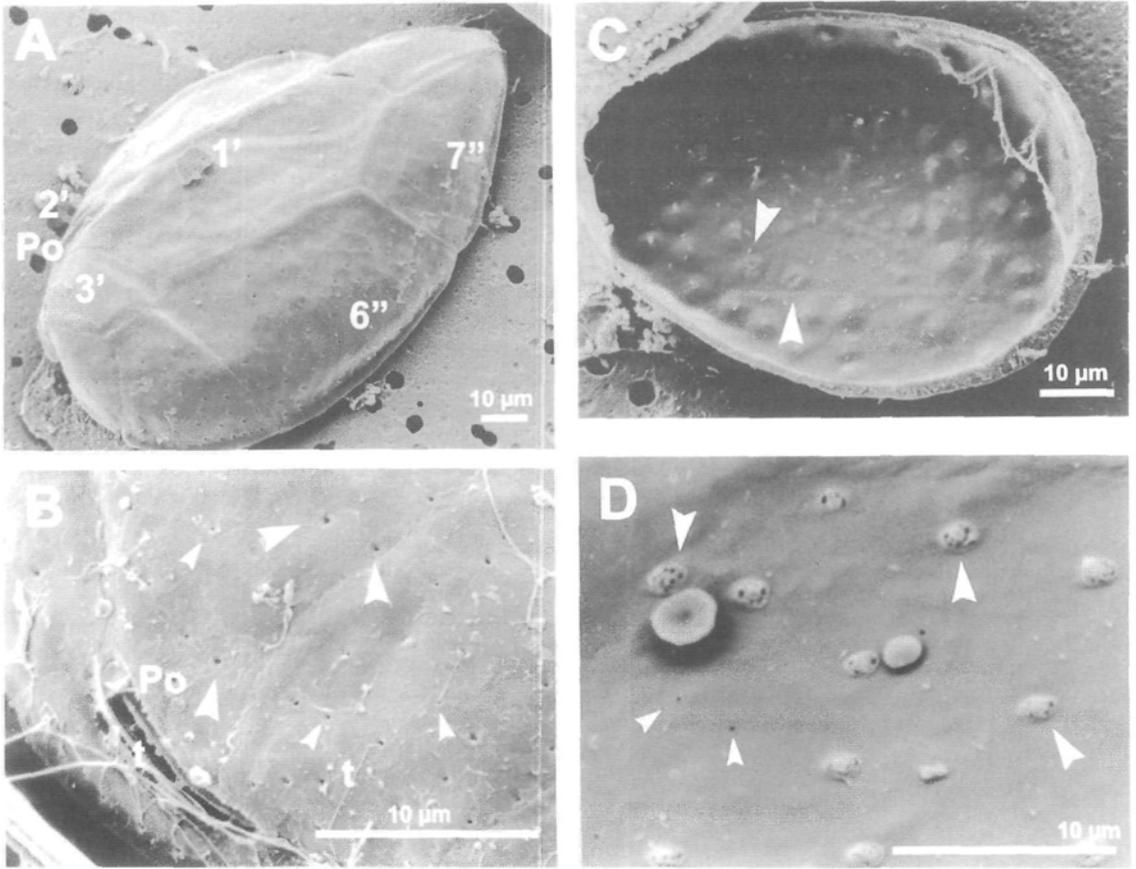


Fig. 3 A, Apical view of *Ostreopsis siamensis*; B, long, curved apical pore (Po) and the small (small arrowhead) and large pores (large arrowhead) with trichocysts (t) on thecal plates; C, inner surface of a separated thecal plate with multipore structure (arrowheads); and D, small pores (small arrowheads) and large multipore structure (large arrowheads) on the inner thecal wall.

small, simple round pores, there are also large multipore structures which are directly linked to large pores on the cell surface (Fig. 3D).

Ostreopsis lenticularis Fukuyo

Cells of *Ostreopsis lenticularis* vary from lenticulate to broadly oval-shaped and slightly pointed ventrally (Fig. 4A–C); 70–95 µm in dorsoventral diameter and 55–75 µm in transdiameter. The shape and morphology of thecal plates of *O. lenticularis* are similar to that of *O. siamensis* except they are large, and in some specimens wider than those of the latter species. Only one type of trichocyst pores is found on surface of *O. lenticularis* cells (Fig. 4D).

Ostreopsis ovata Fukuyo

Cells of *O. ovata* are narrow and ovoid in shape. In terms of size, they are the smallest of the three species recorded. The cells measured 38–50 µm in dorsoventral diameter and 25–35 µm in transdiameter. In apical view apical pore plate (P_o) generally appears to be a short, almost straight slit (Fig. 5A,B). All thecal plates showed small round trichocyst pores (Fig. 5B).

Fig. 5 A, Apical view of *Ostreopsis ovata* showing a small, narrow cell with plates 1', 2', 3', 6'', 7'', and a short, slightly curved apical pore; and B, short, slightly curved apical pore (Po) with scattered pores (arrowheads) and trichocysts (t) on cell surface.

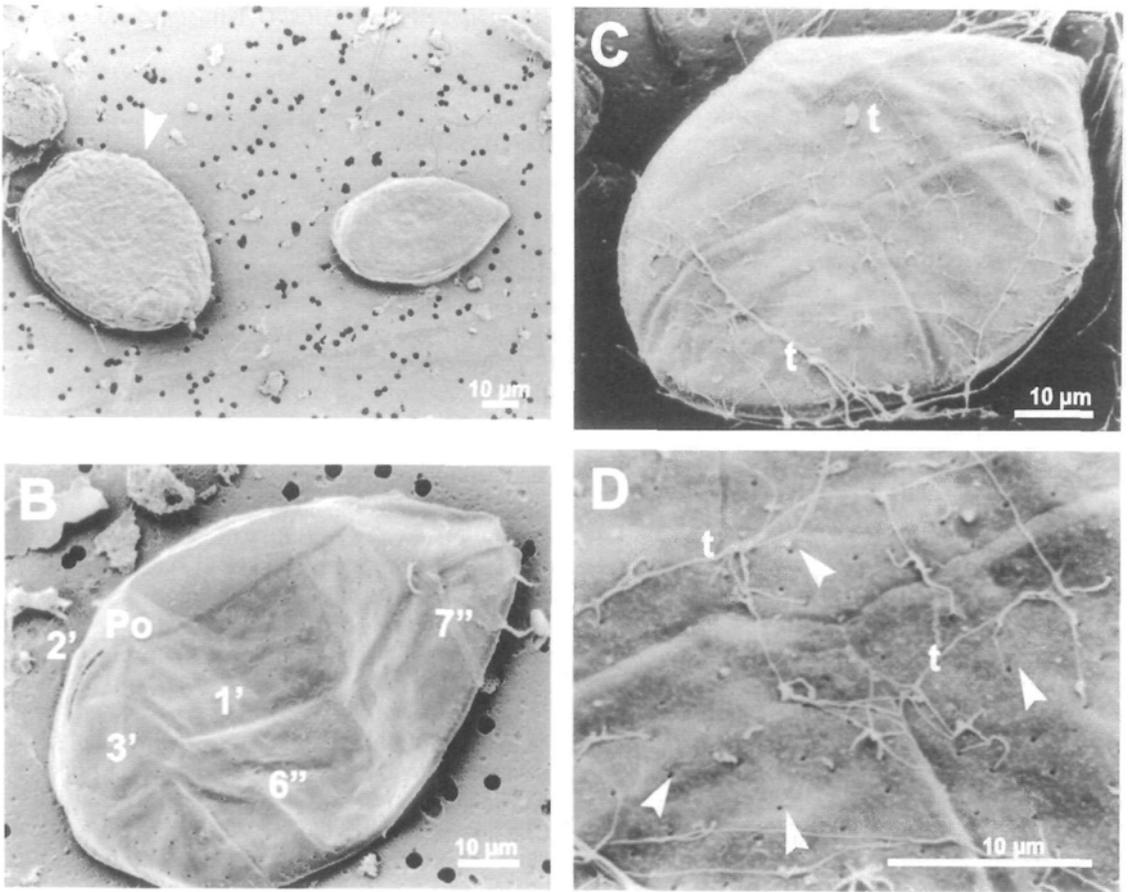
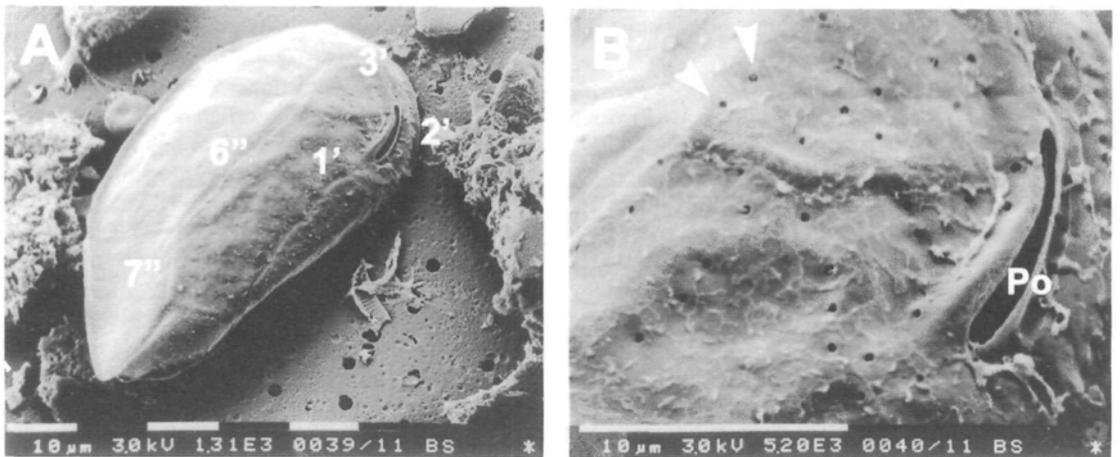


Fig. 4 A, *Ostreopsis lenticularis* cell (arrowhead) on the left, and *O. siamensis* cell on the right; B, apical view of *O. lenticularis* showing the long, curved apical pore (Po) on the left of the cell, and plates 1', 2', 3', 6'', and 7''; C, surface of hypothecal view of a large cell covered with ejected trichocysts (t); and D, surface of thecal plates covered with scattered pores (arrowheads) and trichocysts (t).



Temporal and regional distribution

The three *Ostreopsis* spp.—*O. siamensis*, *O. lenticularis*, and *O. ovata*, were the most common and widely distributed epiphytic dinoflagellate species recorded on both the east and west coasts of the upper North Island particularly in summer (Table 2). Both *Prorocentrum lima* and *Coolia monotis*, however, were less abundant and were quite sporadic in distribution. A very small number of *P. compressum* was also recorded from a sample collected in Rarawa.

In March 1996, highest cell concentrations of *Ostreopsis* spp. (sum of all three species) were recorded on the east coast at both Rarawa (556–1095 cells g⁻¹ FW seaweed) and Tokerau Beach (314–502 cells g⁻¹ FW seaweed) (Table 3). Samples collected from Rangiputa, Cable Bay, Paihia on the east coast, and Scott Pt, The Bluff, and Reef Pt on the west coast showed either very small number of cells (<15 cells g⁻¹ FW seaweed) or in some instances no cells (Table 3). Relatively higher concentrations of *Ostreopsis* spp. appeared to associate with the seaweed *Carpophyllum maschalocarpum* than with the seaweed *Gigartina* sp. collected from Rarawa (Table 3).

In March 1996 samples collected from Tokerau, Rangiputa, Cable Bay, Rarawa on the east coast, and from Reef Pt on the west coast showed *O. siamensis* was dominant and accounted for up to 85% of the total cell concentrations (sum of five epiphytic dinoflagellate species) (Table 4). Both *O. lenticularis* and *O. ovata* were common on seaweeds in the study areas but never built up to high numbers. The proportions of either species generally were quite low and made up 8–38% and 0.5–4% respectively of the total cell concentrations. *P. lima* and *C. monotis* were the least abundant species on those sites in summer. In the following September 1996, only a very small number of *O. siamensis* and *O. lenticularis* were recorded in samples collected from Rangiputa and Rarawa.

In September 1996 *Ostreopsis ovata*, *Prorocentrum lima*, and *Coolia monotis* were absent at all four sites (Table 4). Cell concentrations enumerated in November 1995, October 1996, and April 1997 were more or less in the same range as in September 1996 and were not presented here.

Water samples collected from Tokerau in April 1997 showed only a small number of epiphytic dinoflagellates: *O. siamensis* (0.9×10^3 cells litre⁻¹), *O. lenticularis* (0.1×10^3 cells litre⁻¹), *O. ovata* (0.1×10^3 cells litre⁻¹), and *C. monotis* (0.1×10^3 cells litre⁻¹). Water samples taken from Rarawa showed only a very small number of *O. siamensis*

(0.3×10^3 cells litre⁻¹) and *O. lenticularis* (0.1×10^3 cells litre⁻¹).

DISCUSSION

The sizes, cell morphology, and ornamentation of thecal plates of the three New Zealand *Ostreopsis* spp. generally fit into the descriptions of Fukuyo (1981) and Faust et al. (1996). Under SEM, cells of the New Zealand *O. siamensis* showed randomly spaced surface pores of two different sizes on the theca as were revealed by Faust et al. (1996). These differ from earlier description of *O. siamensis* by Fukuyo (1981) which showed one large pore size only. Moreover, the New Zealand species also showed that the large surface pores on the thecal plates are actually linked to multi-pore structures on the inner face of the thecal wall, whereas the small pores were simple pores on both sides of the cell wall. These pores are apparently trichocyst pores, and numerous trichocysts observed on the surface of thecal plates, presumably had been discharged during preservation.

In this study only medium size trichocyst pores were found on thecal plates of *O. lenticularis*. This is similar to that described by Faust et al. (1996), but is different from earlier description of Fukuyo (1981) which showed pores of two different sizes on thecal plates. Like *O. ovata* observed by both Fukuyo (1981) and Faust et al. (1996), the New Zealand specimens showed one pore size only, and also very delicate thecal plates and generally short, straight apical pore plates (P_o) in apical view.

This study also showed all three *Ostreopsis* spp. were common epiphytic dinoflagellates in northern New Zealand. For the first time these *Ostreopsis* spp. have been reported south of 35°S latitude. It is also clear that in terms of cell abundance there is a regional variation of *Ostreopsis* spp. in northern New Zealand; cell concentrations recorded on the east coast were generally greater than on the west coast. Moderate to high cell concentrations recorded are probably attributable to the less energetic and more stable environments, similar to the Type II system described by Tindall & Morton (1998). Clearly the less energetic conditions are common in such areas as Rangaunu Bay, Doubtless Bay, and Cable Bay on the north-east coast. On the other hand, low cell concentrations recorded on limited samples collected from the north-west coast are probably related to the more energetic areas on open, more exposed sandy beaches (e.g., Scott Pt, The Bluff, Reef Pt) like the

Type I system described by Tindall & Morton (1998).

February and March were the warmest months during the study period. Greatest abundance of *Ostreopsis* spp. (up to 1.1×10^3 cells g^{-1} FW seaweed) occurred in March 1996 on the east coast. This was substantially higher than in November 1995, September/October 1996, and April 1997 in the same areas (generally less than 0.2 cells g^{-1} FW). In summer on the north-eastern New Zealand, sea surface temperatures usually exceeded $21^\circ C$, and were at least $6^\circ C$ higher than in late winter. In the tropical Virgin Islands, only *C. monotis* and *P. lima* were found to be positively correlated with water temperatures, both *O. lenticularis* and *Gambierdiscus toxicus*, however, were negatively correlated with water temperature (Carlson & Tindall 1985). In culture, isolates from tropical and subtropical waters generally exhibit sustained growth between 22 and $31^\circ C$ (see Tindall & Morton 1998).

In March 1996, *Ostreopsis* spp. were associated with almost every species of seaweed collected, with the exception of drifting seaweed collected on two sites off the west coast. The association of these epiphytic dinoflagellates with almost any seaweeds is an indication of their opportunistic behavior in regard to seaweed substrate (Gillespie et al. 1985). On the east coast greater abundance of *Ostreopsis* spp. was consistently recorded at Rarawa. The greatest abundance of *Ostreopsis* spp. was found in association with the seaweed *Carpophyllum maschalocarpum* (1.1×10^3 cells g^{-1} FW), intermediate abundance with a combination of *C. maschalocarpum* and *Ecklonia radiata* (707 cells g^{-1} FW), and the least with *Gigartina* sp. (556 cells g^{-1} FW).

Carpophyllum maschalocarpum is a brown seaweed with small leafy tissues and apparently provides a greater surface area than the larger and less leafy species. Whether there is any active preference by *Ostreopsis* spp. for small and more leafy seaweeds is not clear. In Virgin Islands in the tropics, *O. lenticularis* and *Gambierdiscus toxicus* were found to be more abundant on the brown seaweed *Dictyota* sp. at coral reef stations (Carlson & Tindall 1985), whereas off Queensland coast, Australia, *G. toxicus* was found to be more abundant on *Digenia simplex* (Gillespie et al. 1985).

Cell concentrations of *Ostreopsis* spp. recorded in subtropical northern New Zealand are moderately high compared with those found in the tropics. In tropical regions, cell concentrations of *Ostreopsis* spp. varied from a low of 3 cells g^{-1} FW on *Turbinaria ornata* (Yasumoto et al. 1980), to a

moderate concentration of 308 cells g^{-1} FW on *Heterosiphona gibbesii* (Bomber 1985), and a high of 5.5×10^3 cells g^{-1} FW on *Turbinaria* sp. (Quod pers. comm. see Faust et al. 1996). In Virgin Islands in the Caribbean, a very high concentration of over 21×10^3 cells g^{-1} FW was also recorded on the brown seaweed *Dictyota* sp. (Carlson & Tindall 1985).

In northern New Zealand in summer *O. siamensis* accounted for 64–85% of the total epiphytic flora and is clearly the dominant species. This is in contrast to many tropical regions where *G. toxicus* and *O. lenticularis* were co-dominant, e.g., in Virgin Islands in the Caribbean (Carlson & Tindall 1985), in French Polynesia (Bagnis et al. 1985), and off Queensland coast, Australia (Gillespie et al. 1985). Although *G. toxicus* was recorded only once in a sample collected from northern New Zealand, it was in extremely low concentration, and has never been detected in any other samples collected subsequently from the same areas.

In early spring (October 1996) in the Upper North Island only a very small number of *O. siamensis* and *O. lenticularis* were found, and none of *O. ovata* was recorded. In terms of cell concentrations, even in the more favourable conditions in summer, *O. ovata* usually made up a very small proportion of the total assemblage (<4%). Cell concentrations recorded in the region were also very low (0.5–13 cells g^{-1} FW) in comparison to those recorded at St Leu Reunion, Reunion Island (820, 2122, and 2425 cells g^{-1} FW seaweeds) in the tropics (Faust et al. 1996).

During the study period, cell concentrations of both *P. lima* (3 cells g^{-1} FW seaweeds) and *C. monotis* (0.01 cell g^{-1} FW) recorded were generally very low and did not build up to the same concentration as *Ostreopsis* spp. Cell concentrations of both these species are very low compared with the maxima of $>17 \times 10^3$ and $>1.2 \times 10^6$ cells g^{-1} FW seaweeds of the two respective species reported in Virgin Islands in the Caribbean (Carlson & Tindall 1985). *P. lima* was previously recorded in sediment samples in Rangaunu Harbour (Rhodes & Syhre 1995); cell concentrations recorded in July 1994 were higher (800 cells ml^{-1} sediment) than those recorded on seaweed samples collected in March 1996. *C. monotis* was also reported by Rhodes et al. (1995, 1997) from drift seaweed collected from Ninety Mile Beach, Northland, but no cell concentration was determined in that study.

Ostreopsis spp. also exist as a free-living, "planktonic" form in the water column, particularly in summer, when cell concentrations of these

epiphytes are greatest on seaweeds. In April 1997 a small number of *O. siamensis* (0.9×10^3 cells litre⁻¹) and *O. lenticularis* (0.1×10^3 cells litre⁻¹) were recorded in a water sample taken from Rarawa and Tokerau. This is consistent with observations of free-living *Ostreopsis* spp. made by Faust et al. (1996) in Man of War Cay and South Water Cay, Belire, at Oshigaki Island, Japan, and Reunion Island, north-west Indian Ocean.

Tests conducted on cultures of New Zealand *O. siamensis* showed the presence of a weak palytoxin analog (Rhodes 1997). At this point it is not clear whether there is any link between the lipid-soluble toxins detected in shellfish collected from Rangaunu Harbour and the presence of *O. siamensis* in the areas. In northern New Zealand, in summer, it is likely that the build-up of this species to very high cell concentrations can be a potential source of toxin. Other species such as *P. lima* and *C. monotis* have also tested positive for okadaic acid and unknown polyether compounds respectively (Rhodes & Syhre 1995; Rhodes & Thomas 1997). Potentially *Ostreopsis* can pose a health risk as well. Cultures of *O. lenticularis* and *O. ovata* have yet to be developed. It is not definitely known if any of these species are toxic or not. There is therefore a need for clarification of the toxicity of these *Ostreopsis* species.

ACKNOWLEDGMENTS

We thank Dr J. M. Bradford-Grieve for her constructive criticisms of this manuscript; Dr Y. Fukuyo of the University of Tokyo, Japan, for confirmation of the three *Ostreopsis* spp.; and Mr Arthur Kapa for his assistance to one of us (YS) in collecting plankton samples from Rangaunu Harbour. We also extend our sincere thanks to Dr M. Faust and an anonymous reviewer for comments that improved the manuscript. This work was supported by the New Zealand Foundation of Research, Science and Technology, Contract No. CO1214.

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