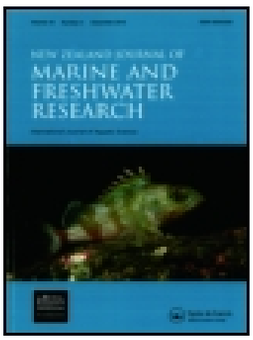

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Short communication

Okadaic acid production by a New Zealand *Prorocentrum lima* isolate

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Abstract Cells of *Prorocentrum lima*, isolated from sediments collected from Rangaunu Harbour, *P. lima* (a Spanish isolate), and *P. compressum*, isolated from wrack collected at Ninety Mile Beach, Northland, New Zealand, were cultured and analysed for okadaic acid by high-performance liquid chromatography (HPLC), using the derivatisation method with ADAM (anthryldiazomethane). *P. lima* (from Northland) was found to be equally toxic as cultures of the known toxic strain *P. lima* (Spanish isolate), and both strains were toxic to *Artemia salina*. *P. compressum* was not toxic to *A. salina* and did not produce okadaic acid.

Keywords *Prorocentrum lima;* okadaic acid; diarrhetic shellfish poisoning; DSP

INTRODUCTION

The benthic dinoflagellate *Prorocentrum lima* (order: Prorocentrales Lemmermann 1910; Chrétiennot-Dinet et al. 1993) has a worldwide distribution. Okadaic acid production by this species has been shown for a number of geographically separated strains, e.g., from Japan (Murakami et al. 1982) and Spain (Lee et al. 1989). The toxin, a polyether fatty acid, causes diarrhetic shellfish poisoning (DSP: Morton & Bomber 1994). To date, the only correlations between DSP in New Zealand shellfish and microalgae have involved the genus *Dinophysis* (L. MacKenzie, pers. comm.). As *Dinophysis* has not been cultured as yet, toxin analyses have been carried out (by HPLC as described in the Methods) on cells isolated directly from freshly collected sea water samples.

Prorocentrum lima has been observed in New Zealand waters, in Northland and the Marlborough Sounds (Smith et al. 1993; Chang 1993; pers. obs.), but has not been tested for toxicity. In this study *Prorocentrum* cells were isolated from Rangaunu Harbour sediments and from wrack collected from Ninety Mile Beach, in Northland, and identified as *P. lima* (Dodge 1975; Faust 1991) and *P. compressum* (Dodge 1975), respectively. Cells were cultured, tested for toxicity using an *Artemia salina* bioassay, and analysed by HPLC for okadaic acid.

METHODS

Microalgae

Prorocentrum lima was isolated from sediments collected in sterile plastic bottles from the Pukewhau Channel in Rangaunu Harbour, Northland (35°10′S; 173°20′E), July 1994 (for the Marine Biotoxin Surveillance Unit), and *P. compressum* was isolated from washings of wrack collected off Ninety Mile Beach, Northland (35°10′S; 173°15′E; collected by Vic Hensley for transfer clearance by MAF Fisheries, Wellington), June 1994. The wrack mainly comprised the macroalgal species *Osmundaria colensoi, Pterocladia lucida, Gigartina* sp., *Glossophora kunthii* and *Landsburgia quercifolia.*

Prorocentrum lima cells were obtained by washing the sediments through a graded series of sieves (beginning with 120 µm through to 30 µm mesh) with filtered synthetic sea water (Seasalts; Sigma) and retaining the final fraction in settling chambers for isolation of microalgal cells. The dinoflagellates were then picked out with modified Pasteur pipettes under an Olympus IMT–2 inverted light microscope. *P. compressum* was obtained by washing wrack in synthetic sea water and then

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treating the washings in the same way as the sediments. *P. compressum*, *P. lima*, and *P. lima* (from Spain), obtained from the University of British Columbia, Canada (NEPCC SP), were cultured in GP medium (Loeblich & Smith 1968) in tissue culture flasks (Nunclon), harvested on glass microfibre filters (Whatman, GF/C) and frozen until required for analysis. Incubation was at 18°C (± 1°C), photon flux 100 μmol m^{-2} s^{-1}, and light : dark regime 14 : 10h.

Identification of *P. lima* and *P. compressum* was by scanning electron microscopic investigation of the surface of the thecae (Cambridge Stereoscan Mark 3; EM Unit, HortResearch, Palmerston North, New Zealand). Cells were passed through an ethanol series before critical-point drying and mounting on metal stubs for gold coating.

Artemia salina toxicity bioassay

Cultured *P. lima* (from Rangaunu and Spain) and *P. compressum* cells were assayed for toxicity using the standard brine shrimp bioassay. The *Artemia* were treated with 0, 5, 50, or 200 *Prorocentrum* cells (in triplicate) and observed over 24 h (Persoone & Wells 1987; Rhodes et al. 1993).

Okadaic acid analysis

Filtered cell cultures were prepared after Morton & Bomber (1994) for derivatisation with ADAM (anthryldiazomethane), followed by high performance liquid chromatography (HPLC) (van Trijp et al. 1994) using a C18 Econosphere (Alltech) column (5 μm; 250 × 4.6 mm), with an acetonitrile: methanol:water eluent (8:1:1), flow rate 1.0 ml min^{-1} and fluorescence detector (Hitachi; excitation 365 nm and emission 412 nm). The okadaic acid standard was 25 μg ml^{-1}.

RESULTS AND DISCUSSION

Investigation of sediments from Rangaunu Harbour, July 1994, revealed the presence of *Prorocentrum lima* (0.8×10^3 ml^{-1} sediment), *Alexandrium ostenfeldii* cysts (5.2×10^3 ml^{-1} sediment), and a mixed population of benthic diatoms ($> 140 \times 10^3$ ml^{-1} sediment). Wrack collected from Ninety Mile Beach, June 1994, bore abundant numbers of *P. compressum* ($\leq 20 \times 10^3$ kg^{-1}), cysts of the dinoflagellates *Scrippsiella* and *Protoperidinium*, and the surf diatom *Gonioceros armatum*.

Prorocentrum compressum and *P. lima* (from Spain) were readily cultured in GP medium, with a total of 24×10^4 and 30×10^6 cells, respectively, being harvested after 10 weeks. Cells of *P. lima* (from Rangaunu) initially grew slowly, with a total of 20×10^5 being harvested from an original single cell after 18 weeks. The original light microscope identifications of the species were confirmed by scanning electron microscopy of the thecal plates to determine their anterior structure and the form and distribution of trichocyst pores (Dodge 1975).

Prorocentrum lima (both strains), but not *P. compressum*, was toxic to *Artemia*. Neither five *P. lima* cells nor the controls caused death of *Artemia*, but 50 cells caused a 50% death response in 24 h and 200 cells a 50% death response in 20 h. Stationary phase cultures of *P. lima* (both strains) were equally toxic, c. 5 pg okadaic acid $cell^{-1}$ (Table 1); *P. compressum* did not produce okadaic acid. The toxin concentration determined for *P. lima* (Rangaunu) was within a similar range to concentrations previously determined for another Spanish isolate (5–26 pg $cell^{-1}$) and a Japanese strain (4 pg $cell^{-1}$) of *P. lima* (Lee et al. 1989; Murakami et al. 1982). Previous analyses of *P. lima* (from Spain) indicate that nitrogen- and phosphate-limited cultures in stationary phase are more toxic than nutrient-replete or exponential-phase cultures (Haecky 1991).

Initially *P. lima* (from Rangaunu) was difficult to isolate as the cells attached by their flagella to the isolation chambers and culture vessel surfaces. The Pukewhau Channel, from which *Prorocentrum* was isolated, is subjected to constant flushing by tidal movements. The ability of *P. lima* to attach firmly by its flagella to surfaces might aid in its ability to withstand water movements, by binding it into the microalgal surface mat. The presence of an okadaic acid producing dinoflagellate, firmly established in Rangaunu Harbour sediments, warrants further investigation to determine whether this species helps maintain the integrity of the surface layer and thus minimise sediment resuspension. If this is so, it might explain the general absence of diarrhetic shellfish toxin (DST) in oysters farmed in the harbour. *Berkeleya rutilans*,

Table 1 Production of okadaic acid by *Prorocentrum* species isolated from Northland, New Zealand.

Microalgal species	Okadaic acid pg $cell^{-1}$ (±1.0 pg)
Prorocentrum compressum	0
P. lima (Rangaunu isolate)	6.3
P. lima (from Spain)	4.0

a mat-forming diatom that occurs in Tory Channel, Marlborough Sounds, has such a sediment-binding ability, which provides ecological stability in an area subject to strong water movements (Maxwell 1993).

Rangaunu Harbour is surrounded by mangroves and consists largely of exposed mudflats at low tide. *Prorocentrum* species assemblages, including *P. lima*, can constitute a significant proportion of (Faust 1990; 1991, 1993), and major primary producer within (Carlson & Tindall 1985), the total dinoflagellate population of mangrove habitats. At Rangaunu Harbour *Alexandrium ostenfeldii* (cyst form) was the only co-dominant dinoflagellate. It would be important to determine whether *P. lima* is a permanent and dominant species within the mixed sediment populations or whether it has a sporadic or seasonal presence.

Although *P. lima* produces okadaic acid, this dinoflagellate species has not been linked to DST in New Zealand. To date, in New Zealand, all correlations of microalgal cells *in vivo* with DST have involved two planktonic species, *Dinophysis acuta* and *D. acuminata* (L. MacKenzie, pers. comm.), neither of which has been cultured and analysed for DST in vitro. The presence of this benthic toxin producer suggests that phytoplankton monitoring as an assessment of shellfish biotoxin risk in areas where bottom-feeding wild shellfish are being harvested will not be adequate; sediment analyses for toxic microalgae or shellfish flesh testing for biotoxins will be required in such areas.

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