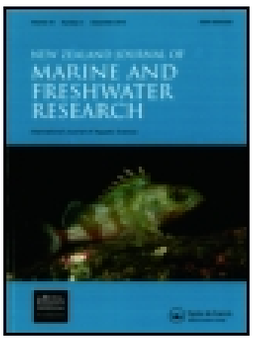

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Coolia monotis (Dinophyceae): a toxic epiphytic microalgal species found in New Zealand (Note)

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Abstract *Coolia monotis* Meunier (Dinophyceae) was isolated from drift seaweed collected from Ninety Mile Beach, Northland, New Zealand. It is a new record for New Zealand waters. The microalga was identified by scanning electron microscopy and cultured in a seawater-based medium. Cultures grew preferentially at 25°C (subtropical temperatures), rather than at 20°C (temperate), and at salinities >28. *Coolia monotis* proved to be toxic to larvae of *Artemia salina* and *Haliotis virginea*.

Keywords *Coolia*; epiphytic dinoflagellate; cooliatoxin; yessotoxin

INTRODUCTION

Coolia monotis was first described by Meunier (1919) in oyster beds, and more recently (Fukuyo 1981) as an epiphyte on seaweeds. *C. monotis* was isolated from the macroalga *Cladophora* sp., collected from Platypus Bay, Queensland, Australia in 1988 (Holmes et al. 1995), and the genus *Coolia* was recently reported on drift macroalgae in Rangaunu Harbour and Doubtless Bay, Northland, New Zealand (Chang 1996). *Coolia monotis* produces a novel toxin, cooliatoxin, which might be a mono-sulphated analogue of yessotoxin (Holmes et al. 1995).

Oyster (*Crassostrea gigas*) harvesting has been restricted in Rangaunu Harbour for considerable periods over the last few years. The restrictions have largely been due to the detection of low levels of unidentified lipid soluble toxins, which have elicited neurotoxic shellfish poisoning-like symptoms in mouse bioassays (New Zealand Marine Biotoxin Management Board records). The mouse response was not typical for brevetoxin, and yessotoxin (Murata et al. 1987) was therefore considered a possible candidate for the toxin in the Rangaunu Harbour shellfish. Identification, culturing, and toxicity testing of new microalgae from Northland is important for the determination of the source of the ongoing shellfish toxicity. This paper reports that *Coolia monotis* has been isolated from Northland for the first time. The organism has been cultured and tested positive in toxicity assays with *Artemia* and *Haliotis* larvae.

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METHOD

Microalga: isolation, culture, and identification

Coolia monotis (CAWD39) was isolated by micropipette as a single cell from washings of wrack collected off Ninety Mile Beach, Northland (35°10′S; 173°15′E), February 1995. The wrack comprised mainly foliose red and smaller brown algae (e.g., *Landsburgia quercifolia*).

The dinoflagellate was grown in GP medium (Loeblich & Smith 1968) under standard microalgal growth conditions (Rhodes & Syhre 1995). Established cultures were grown in quadruplicate at both 20°C and 25°C, and at salinities of 15 to 34 (practical salinity scale), to indicate optimal growth conditions. Growth was measured by chlorophyll *a* increases using a Turner Designs Fluorometer (Model 10-005R), with filter combination for *in vivo* fluorescence. ANOVA (combined with Tukey-Kramer method) was used to determine the significance of the results. Scanning electron microscopy (SEM; Cambridge Stereoscan Mark 3; EM Unit, HortResearch, Palmerston North, New

Zealand: Rhodes & Syhre 1995) was used to identify the isolate.

Toxicity bioassays

Coolia monotis (c. 1000 cells per well) was assayed for toxicity using both the standard brine shrimp bioassay (Persoone & Wells 1987; Rhodes & Syhre 1995) and a paua larval (*Haliotis virginea*) bioassay. In the latter, 20 larvae per tissue culture plate well were treated in the same way as *Artemia salina*, with all assays carried out in triplicate. A control of media addition alone and a non-toxic algal control (*Gymnodinium* sp.) were also tested. Morbidity in *Artemia* was scored as "+" (brine shrimps twitching on bottom of well) or "++" (*Artemia* immobile). Morbidity in paua larvae referred to settling of larvae on bottom of well, circling movements without swimming, and the withdrawing of the larval body away from the shell. The 10-day old paua larvae were obtained from the Glenhaven Aquaculture Centre, Nelson, New Zealand and *Artemia* eggs from a local pet store.

RESULTS AND DISCUSSION

A clonal culture of the golden-brown dinoflagellate *Coolia monotis* was isolated from drift macroalgae collected from the sands of Ninety Mile Beach, Northland, in summer 1995. It is not clear whether the alga was originally attached to the seaweed or existed as a sand-dweller. Under the light microscope the cells appeared lens shaped, as in Fukuyo's (1981) discussion of *C. monotis*, and two size ranges of cultured cells were observed (19.6–25 μm width × 25–30 μm length and 30 μm width × 35–40 μm length). The phenomenon of small cells was previously observed in the life history of *Coolia monotis* (Silva & Faust 1995). The New Zealand isolate was smaller than described by Faust (1992), and had some similarities to *C. tropicalis* (Faust 1995), in particular the sparsely scattered thecal pores. However the apical pore, a 7μm slit surrounded by evenly spaced pores, was similar in shape and length to that described for *C. monotis* (Steidinger & Tangen 1996). The identification was made primarily on the basis of the shape and arrangement of the thecal plates, in particular apical plate 1' and precingular plate 7" (Faust 1992, 1995; Steidinger & Tangen 1996; Maria Faust pers. comm.). Plate 1' was not wedge-shaped, as is characteristic for *C. tropicalis*.

Cultures of *C. monotis* grew faster at 25°C than at 20°C ($P < 0.05$) although, as has previously been described (Faust 1992), the doubling times were extremely slow (4 days at 25°C, 10:14 h dark:light cycle; 100 μmol photons m^{-2} s^{-1}). *C. monotis* did not grow at salinities < 20.

Toxicity was indicated by the results of the *Artemia salina* and paua larval bioassays (Table 1). Sixty-two percent of the *Artemia* became morbid (+) at 8–12 h; 16% dead and 70% morbid (++) at 24 h. Fifty percent of the paua larvae were morbid at 1.5 h; 100% by 24 h. The media and non-toxic microalgal controls had no effect on the viability of the bioassay organisms.

Coolia monotis, isolated from Queensland, Australia, was initially thought to be non-toxic. Holmes et al. (1995) have recently described cooliatoxin (a mono-sulphated polyether toxin) from this species, suggesting that it might be an analogue of yessotoxin. Desulphation of yessotoxin, which has two sulphate esters (Murata et al. 1987), reduces its lethality to mice (Nagai et al. 1990). If cooliatoxin is less toxic to mice than yessotoxin, then low levels in microalgae could lead to toxic species being overlooked. Further work will be carried out to characterise the toxic factors indicated by the toxicity bioassays of the New Zealand isolate of *C. monotis*.

Table 1 *Artemia salina* and *Haliotis virginea* bioassays: time (h) taken to elicit 50% morbidity (tM_{50}). A non-toxic *Gymnodinium* species and growth medium were used as controls.

	Artemia		*Haliotis*
Microalgal species	tM_{50}+	tM_{50}++	tM_{50}+
Coolia tropicalis	**8.0**	**<24.0**	**1.5**
Gymnodinium sp.	>72.0	>72.0	>72.0
Control (medium)	>72.0	>72.0	>72.0

tM_{50}+: morbidity; tM_{50}++: immobility (but not death). Triplicate bioassays, each with 10 *Artemia* or 20 paua larvae, were carried out with stationary phase microalgal cultures (c. 1000 cells per well; GP medium; 18°C; 100 μmol m^{-2} s^{-1}; 10:14 h dark:light). Bioassays incubated under same conditions as algal cultures.

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