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Benthic dinoflagellate toxins in two warm-temperate estuaries: Rangaunu and Parengarenga Harbours, Northland, New Zealand

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ABSTRACT

The analysis of a variety of environmental samples and the installation of passive solid phase adsorption devices (SPATT) in two warm-temperate estuaries (Rangaunu and Parengarenga Harbours), during consecutive summers (2009 and 2010), revealed the pervasive influence of bioactive polyether compounds secreted by benthic dinoflagellates within the mangrove and sea-grass habitats. Pinnatoxin (PnTx) analogues PnTx-E and PnTx-F and okadaic acid (OA) and its esters were the most abundant, though traces of other polyether compounds (dinophysistoxins, pectenotoxin, spirolides), were also detected. In sediments, algal mats and micro-algal films, the parent compound PnTx-F was the predominate analogue. In bivalves and gastropods PnTx-E and PnTx-F were either present in equivalent amounts or there was a predominance of the former, indicative of *in vivo* metabolism. Esterified OA was the predominant form of this toxin in the deposit feeding sea hare *Bursatella leachii*, however OA-toxins were only about 14% as abundant as the combined PnTx analogues in this animal. Conversely levels of PnTxs accumulated within the SPATT bags were only 50% of total-OA, about 10% of which was in the form of OA-esters. Neither OA nor OA-esters were observed in the oyster *Crassotrea gigas*. The levels of total-PnTxs in *C. gigas* were about 8% of that observed in *B. leachii* (200 and 2580 μg/kg respectively). Although there was abundant OA in this environment, cultured oysters did not incorporate this toxin presumably because they were not exposed to *Prorocentrum lima* cells in the water column or were unable to digest these cells. The low levels of pinnatoxins sequestered by oysters in these estuaries, despite persistent and very abundant populations of the causative dinoflagellate, is probably also due to the general inaccessibility of these cells to the shellfish and therefore there appears to be a low risk to human consumers

1. Introduction

Rangaunu and Parengarenga Harbours (Fig. 1) are large (97 km^2 and 63 km^2 respectively) shallow estuaries in the far north of the North Island of New Zealand that contain extensive mangrove (*Avicennia marina* var *australasica*) and sea-grass (*Zostera capricorni*) habitats. In Rangaunu Harbour the mangroves occupy about 30 km^2 and comprise the largest contiguous stand in New Zealand, while the sea-grass meadows occupy a further 20 km^2 (May, 1999). These inlets have international significance as habitats for migratory waders and are important fin-fish nurseries (Morrison et al., 2007). There are several commercial Pacific oyster (*Crassotrea gigas*) intertidal rack-culture growing operations in both estuaries.

During the 1990s, mouse assay screens for lipid soluble toxins, conducted during a nation-wide shellfish-biotoxin monitoring programme, frequently revealed the presence of an unidentified toxin (referred to as "Rangaunu Harbour toxin") in oysters from the Rangaunu Harbour, although there was no evidence of any human illness resulting from consumption of these shellfish (Hay et al., 2000). Because of these positive mouse assays and the unknown nature of the toxicity, commercial oyster farming ceased for several years and only recommenced in mid 2000 when the mouse assay screen test was no longer used and chemical (LC–MS) testing became established. This confirmed that known regulated toxin groups such as the DSP-toxins and brevetoxins were not present in the shellfish. Recently however, as the result of a comparison of Rangaunu Harbour oysters with mouse-assay positive oysters from Australia, the identity of the previously unknown mouse-assay toxicity was discovered (McNabb et al., 2008; Selwood et al., 2010) to be due to low levels of several new pinnatoxins analogues (PnTx-E–G; Fig. 2).

To identify the source of pinnatoxins, and document other micro-algae derived toxins that exist in the environment of the northern estuaries, samples of a variety of biological material were

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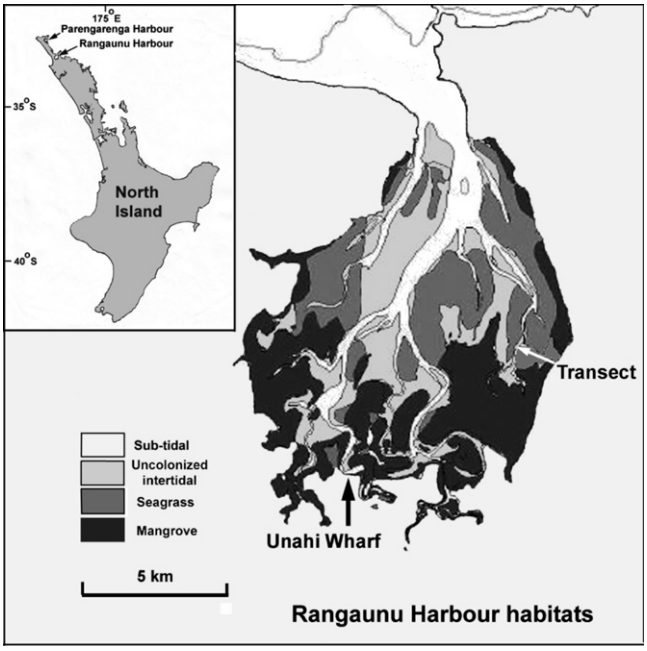

Fig. 1. Geographic location of Rangaunu and Parengarenga Harbours, Northland, New Zealand and macrophyte habitats and the location of the SPATT sampling transect in Rangaunu Harbour.

obtained, and solid phase adsorption toxin tracking (SPATT) devices (MacKenzie et al., 2004) were deployed, on two occasions during February 2009 and 2010. The analysis of these samples was carried out by LC–MS/MS and the results are reported here. The study that led to the identification, isolation and culture of the pinnatoxin producer from the estuary is reported elsewhere (Rhodes et al., 2010).

2. Methods

2.1. Sampling

The collection of environmental samples from Rangaunu Harbour took place on three occasions: 23–25 February 2009, 28–29 March 2009 and 24 February–2 May 2010 and from Parengarenga Harbour during the latter period only.

During the first Rangaunu Harbour sampling SPATT bags (MacKenzie et al., 2004) containing approximately 4 g dry weight of HP20 resin within a 80 μm polyester mesh bag were placed within "Clam" samplers that were mounted on pedestals 100 mm above the sediment surface (Fig. 3.). The "Clam" samplers were designed to simulate the position of oysters on the inter-tidal racks and keep the SPATT bags immersed in seawater, and protected from the effects of exposure to wind and sunlight, during low tide periods. The samplers consisted of a plastic box with a hinged lid. Attached beneath the lid was a removable cage that contained the SPATT bag and on top a block of high density polystyrene. As the tide rose the buoyant lid opened exposing the SPATT bag to the seawater, when the tide ebbed the lid closed and the SPATT bag was immersed in the water remaining in the box. Six "Clam" samplers were placed at 50 m intervals along a transect (Fig. 1) from the margin of the mangroves across the sea-grass meadows to just above the level of standing water at low tide. The samplers were in place for 48 h over four high-tide cycles. It is not known for certain how long the SPATT bags were submerged though those highest on the shore (site 1) would have been submerged for less time than those lower down (site 6). In addition to the across shore transect, SPATT bags were placed in a small pool within the mangroves near the transect, and about 5 km away suspended from a wharf in the brackish water environment at the mouth of the Unahi River.

During the summer 2010 sampling, SPATT bags were anchored within shallow pools at three locations in Rangaunu Harbour including the open sea-grass meadows (A), on cyanobacterial mats

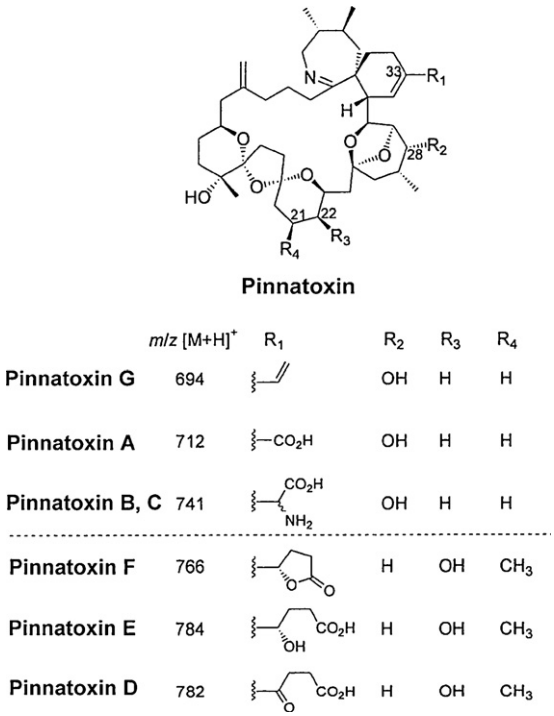

Fig. 2. Molecular structures of pinnatoxins found in Rangaunu and Parengarenga Harbours.

Fig. 3. 'Clam' sampler with the buoyant lid held open. The SPATT bag was placed in the plastic cage under the lid of the sampler.