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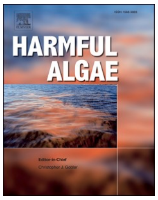

Sub-tropical benthic/epiphytic dinoflagellates of Aotearoa New Zealand and Rangitāhua Kermadec Islands

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ABSTRACT

Temperatures and temperature anomalies have been increasing in the sub-tropical regions of Aotearoa New Zealand and these changes may impact on harmful algal bloom (HAB) events. Benthic and epiphytic dinoflagellates, particularly the toxin producers, are the focus of this study as it is predicted that under future climate conditions they may produce more toxins or marine animals may become more susceptible to them. The results of past expeditions to Rangitāhua Kermadec Islands and sampling trips to Northland, Aotearoa New Zealand, are summarised and the results of the most recent trips to both regions are presented. The macroalgal habitats of the dinoflagellates are also characterised. Dinoflagellate species not previously identified in Rangitāhua include *Coolia canariensis, C. palmyrensis,* and *C. tropicalis,* all identified by DNA sequencing of the large subunit ribosomal RNA region. *Gambierdiscus polynesiensis* was again isolated and produced 44-methylgambierone and gambierone, and one isolate produced ciguatoxins, the cause of Ciguatera Poisoning. An *Ostreopsis tairoto* isolate, as analysed by the oxidative cleavage method, produced a palytoxin (PLTX)-like amine oxidation fragment, but when analysed for PLTX-like analogues using a new intact method none were detected indicating an 'unknown' PLTX-like compound is produced by this isolate. Isolates of *O.* cf. *siamensis* (*Ostreopsis* sp. 9), collected in Northland, were also analysed using the oxidative cleavage method, with the common PLTX-like amine fragment and the amide fragment corresponding to bishomoPLTX detected in all isolates. Again, the intact method indicated no detections in the isolates, again suggesting an unknown compound was being produced by these isolates. *Prorocentrum hoffmannianum* isolates produced okadaic acid (OA) and isoDTX-1 and *P. lima* isolates produced OA, DTX-1, and isoDTX-1. It is expected that new species of potentially harmful, benthic dinoflagellates will continue to be recorded in Aotearoa New Zealand and the results from Rangitāhua provide a guide to the HAB species to expect in sub-tropical Northland as the oceans continue to warm.

1. Introduction

Aotearoa New Zealand's coastline covers 15,000 km and the territorial seas cover nearly 170,000 km^{2}s. If the exclusive economic zone (EEZ) is included this takes the area to >5.8 million square kilometres. Oceans are suffering environmental degradation and imminent threat of extinction for some species (Severinsen et al., 2021) and marine heatwaves now occur regularly in the Tasman Sea and Pacific Ocean (Salinger et al., 2019; Sutton and Brown, 2019). New Zealand's coastal waters have been warming at nearly four times the global average rate (Oliver et al., 2017) and the changes may impact microalgal species that form harmful algal blooms (HAB), particularly sub-tropical dinoflagellate species (Hallegraeff, 2010; Ministry for the Environment, 2018; Rhodes et al., 2020; Dai et al., 2023).

As highlighted by Rolton et al. (2022), increased temperatures and temperature anomalies affect metabolic and physiological responses of important fish and shellfish species, making them more susceptible to the effects of HABs. Benthic and epiphytic dinoflagellates, particularly

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the toxin producers, are an important focus of current marine research as they may either produce more toxins or marine animals may become more susceptible to them. Filter feeding shellfish can sequester a range of suspended material (for example, heavy metals, pathogenic bacteria, and viruses), and this includes toxic dinoflagellates.

1.1. Rangitāhua Kermadec Islands (Rangitāhua)

Rangitāhua is at the northern limit of New Zealand's territories (Fig. 1) and the creation of a Kermadec/Rangitāhua Ocean Sanctuary is under discussion. It will cover 620,000 km^{2}s and will be one of the world's largest marine reserves. Discussions with Ngāti Kurī, a Māori iwi from Northland, New Zealand, continue as the rohe (tribal area) of the iwi includes Rangitāhua as well as the most northern tip of the North Island. The islands are approximately 1000 km northeast of New Zealand and are uninhabited. They comprise a chain of submerged volcanoes and an ocean trench reaching depths of 10 km (Fig. 1).

The first expedition to Rangitāhua from which dinoflagellates were isolated was on board RV Braveheart in November 2013. During that trip, samples were collected from coral surfaces and mixed macroalgae at the Milne Islets, near Boat Cove, Raoul Island (Fig. 1). Raoul Island is the largest and northernmost of the main islands (latitude/longitude: 29°16′S, 177°55′W) and at that time *Gambierdiscus* sp., *Ostreopsis* sp. 3, *Coolia malayensis, Amphidinium massartii, Prorocentrum lima* and *P.* cf. *emarginatum* were isolated. The *Ostreopsis* species produced low concentrations of palytoxin (PLTX) equivalents as determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Table 1; Rhodes et al., 2014a, 2015). *Ostreopsis* sp. 3 has now been described as *Ostreopsis tairoto* Verma, Hoppenrath, Smith, Rhodes, et Sh.Murray sp. nov. (Verma et al., 2022).

The next expedition was carried out in November 2015, again aboard RV Braveheart, and sampling was carried out on the west side of North Meyer Island (Fig. 1). A novel species, *G. honu,* was isolated from non-geniculate corallines and produced maitotoxin (MTX)-7 (Murray et al., 2022) as well as 44-methylgambierone (44-MG; previously known as MTX-3). The more prevalent *G. australes*, which produces MTX-1 and 44-MG (Murray et al., 2019) was isolated from macroalgae in the genera *Spyridia, Asparagopsis, Dasya, Microdictyon* and *Caulerpa* (Rhodes et al., 2017a, 2017b, 2017c; Smith et al., 2017a). Dominant species detected by high throughput sequencing metabarcoding (HTS) included *A. carterae, C. malayensis, P. hoffmannianum*, and *Ostreopsis tairoto*. The latter again produced low concentrations of PLTX-like compounds (Smith et al., 2017a). An *Alexandrium* taxon associated with macroalgae (designated *Alexandrium* sp. type 2; Nishimura et al., 2021) was also detected by HTS analysis (Smith et al., 2017a). Although a Japanese *Alexandrium* sp. type 2 strain showed toxicity by mouse bioassay (Nishimura et al., 2021), no strains have yet been established from Rangitāhua and therefore their toxin production and toxicity are currently unknown.

In November 2016 sampling was carried out by divers on the southeast coast of Macauley Island (Fig. 1), and *G. australes* and *G. polynesiensis* isolates were analysed for ciguatoxins (CTX) and MTXs. *Gambierdiscus australes* had an average of 3–36 pg/cell MTX-1 per cell ($n = 25$) and *G. polynesiensis* ($n = 2$) produced trace levels of CTX (Munday et al., 2017; Rhodes et al., 2017d). *Gambierdiscus* species tested have all produced 44-MG (Murray et al., 2019, 2020, 2022) and some *Gambierdiscus* species also produce gambierone (Murray et al., 2021). The dinoflagellates *Ostreopsis tairoto* (a non-toxic isolate), *C. malayensis,* and isolates in the genera *Prorocentrum* and *Amphidinium,* were also identified. Sampling was also carried out at L'Esperance Rock, the

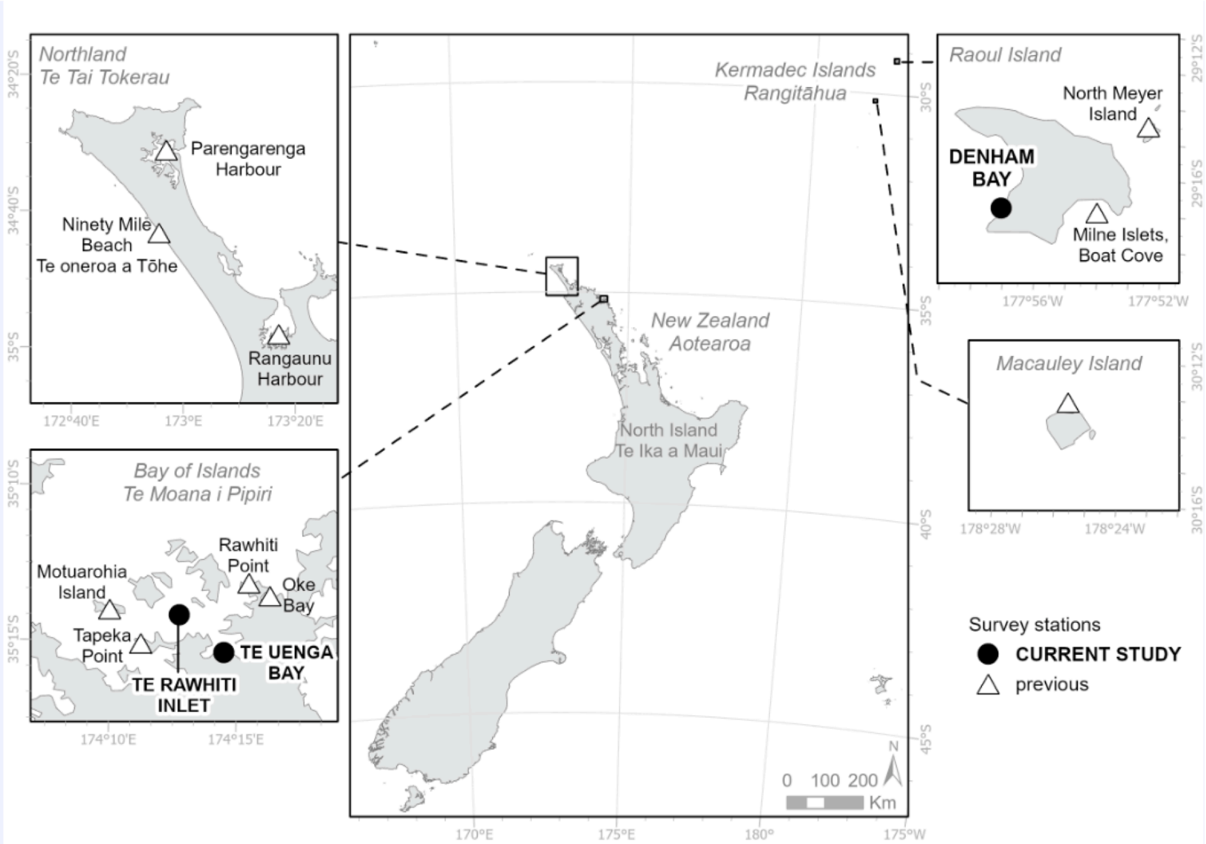

Fig. 1. Maps of Northland, Aotearoa New Zealand and Rangitāhua Kermadec Islands showing sites referenced in this paper. Denham Bay, Raoul Island, the site where macroalgae were sampled for epiphytic dinoflagellates during the expedition in November 2021, is highlighted. In Northland, the November 2021 sampling sites were Te Uenga Bay and Te Rawhiti Inlet.

Table 1
Selected epiphytic and benthic dinoflagellate isolates, representing species collected in Rangitāhua Kermadec Islands, many maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM). Isolates from expedition in 2021 in bold.

Species	CICCM CAW code	GenBank code	Macroalgae hosts[1]	Sites[2]	Toxins LC-MS/MS	Expedition*
Amphidinium carterae	NR	NR	Mixed macroalgae	North Meyer Is	NT	2015
	NR	NR	*Dictyota* aff. *pfaffi*	**Denham Bay, Raoul Is**	NT	**Nov 2021**
cf. *massartii*	D222	KM259619	Mixed macroalgae	Milne Islets (near Raoul Is)	NT	2013
	D377	**OQ572747**	*Caulerpa racemosa*	**Denham Bay, Raoul Is**	**NT**	**Nov 2021**
Coolia canariensis	**D386, D387**	**OQ572753, OQ572754**	*Distromium* sp.	**Denham Bay, Raoul Is**	**Nil**	**Nov 2021**
C. malayensis	D214	NR	Mixed macroalgae	Boat Cove, Raoul Is	44-MG	2013
	NR	NR	Mixed macroalgae	Macauley Is	NT	2015
	NR	NR	Mixed macroalgae	North Meyer Is	NT	2015
C. palmyrensis	**D385**	**OQ572752**	Cyanobacteria undetermined, *Distromium* sp., *Asparagopsis taxiformis, Caulerpa webbiana*	**Denham Bay, Raoul Is**	**Nil**	**Nov 2021**
C. tropicalis	**D384, D388**	**OQ572751, OQ572755**	*Distromium* sp., *Caulerpa webbiana*	**Denham Bay, Raoul Is**	**44-MG**	**Nov 2021**
Gambierdiscus australes	D245, D246, D255, D256	MF109033	Mixed macroalgae	Macauley Is	MTX-1, 44-MG	2016
	D381	**OQ572749**	*Asparagopsis taxiformis*	**Denham Bay, Raoul Is**	**MTX-1, MTX-5, 44-MG**	**Nov 2021**
G. honu	D242	KY062662, KY062663	Mixed macroalgae	Macauley Is	MTX-7, G, 44-MG	2015
G. polynesiensis	D254, D259	MF109032	Mixed macroalgae	Macauley Is	Trace CTX, 44-MG	2016
	D380	**OQ572748**	*Distromium* sp.	**Denham Bay, Raoul Is**	**Trace CTX, G, 44-MG**	**Nov 2021**
Ostreopsis tairoto	D221	KM360088, KY197854	Mixed macroalgae	Boat Cove, Raoul Is	PLTX equiv.[§]	2013
	D272	NR	Mixed macroalgae	Macauley Is	PLTX equiv. [§]	2017
	D383	**OQ572750**	*Plocamium* sp. *Delisea pulchra*	**Denham Bay, Raoul Is**	**PLTX equiv.** [§]	**Nov 2021**
O. cf. *ovata*	D241	KY069060, KY069061	Mixed macroalgae	North Meyer Is	NT	2015
Prorocentrum cf. *emarginatum*	D228	KM360089	Mixed macroalgae	Boat Cove, Raoul Is	Neg. OA and prorocentrolides	2013
	D392, D395	**OQ572742, OQ572745**	*Distromium* sp., *Actinotrichia* sp., *Dictyota* sp., *Delisea pulchra, Plocamium* sp., *Galaxaura* sp.	**Denham Bay, Raoul Is**	**Nil**	**Nov 2021**
P.* cf. *fukuyoi	**D389, D390, D391, D393**	**OQ572739, OQ572740, OQ572741, OQ572743**	*Dictyota* aff. *pfaffi, Distromium* spp. *Dictyota intermedia*	**Denham Bay, Raoul Is**	**Nil**	**Nov 2021**
P. hoffmannianum	**D396**	**OQ572746**	*Dictyota* aff. *pfaffi, Caulerpa webbiana*	**Denham Bay, Raoul Is**	**OA, isoDTX-1**	**Nov 2021**
P. lima	D283	LC422235	*Dictyocha* sp.	Boat Cove, **Raoul Is**	**OA, DTX-1**	2018
	D394	**OQ572744**	*Dictyota intermedia, Dictyota* sp., *Ganonema farinosum, Delisea pulchra, Plocamium* sp., *Asparagopsis taxiformis*	**Denham Bay, Raoul Is**	**OA, DTX-1**	**Nov 2021**

CICCM: Cawthron Institute Culture Collection of Microalgae; MTX: maitotoxin; CTX: ciguatoxin; G: gambierone; MG: methylgambierone; PLTX: palytoxin; OA: okadaic acid; Nil: No toxins detected; NT: not tested; NR: not registered; *Expeditions organised by Auckland War Memorial Museum: 2013 to Boat Cove, Raoul Is.; 2015 to North Meyer Is.; 2016 to Macauley Is.; 2021 to Denham Bay, Raoul Is.

[§] Identified from the common PLTX-like amine oxidation fragment.

southernmost islet in Rangitāhua, but only pennate diatoms and no dinoflagellates were found in these samples and so results are not reported.

The most recent expedition was in November 2021 (D'Archino et al., 2022) and the results are presented in this study. Further expeditions to Rangitāhua, to be led by Ngāti Kurī and Auckland War Memorial Museum, are planned over the next few years.

1.2. Northland, Aotearoa New Zealand

Research into the epiphytic and benthic dinoflagellates of the subtropical north and north-eastern mainland of Aotearoa New Zealand began several decades ago with the initial focus being on Rangaunu and Parengarenga Harbours and Ninety Mile Beach (Fig. 1). Isolates of *Coolia* were first obtained from drift seaweed (or wrack) collected from Ninety Mile Beach in February 1995. The wrack was collected commercially for the attached Greenshell™ mussel spat and was comprised of rhodophytes (*Osmundaria colensoi, Pterocladia* sp. and corallines) and phaeophytes (for example, *Landsburgia quercifolia*). The *Coolia* isolate was described by scanning electron microscopy as *C. monotis* (Rhodes and Thomas, 1997), a species which has been reported as a cooliatoxin producer (Holmes et al., 1995). More recently the Aotearoa New Zealand isolate, maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM) as CAWD39, was re-classified as *C. malayensis* based on DNA sequencing data (Leaw et al., 2010; Rhodes et al., 2014b). Many more *C. malayensis* isolates have since been obtained from sediments, macroalgae, including *Carpophyllum* sp. at the entrance to Rangaunu Harbour and *Ceratium* sp. in the Bay of Islands, and also seagrass (*Zostera muelleri*) in the Bay of Islands (L. Rhodes, personal observations). Strains of *C. malayensis* were variable in their production of 44-MG (Murray et al., 2019, 2020). The extracts of *C. malayensis* were also variable in their toxicity to mice (administered by intraperitoneal (i. p.) injection), with responses (strain dependant) ranging from no effect to death in three minutes (Rhodes et al., 2010).

Microalgal research in Northland increased following the detection of toxins in Pacific oysters (*Crassostrea gigas*) from Rangaunu Harbour in 2007 and 2008 (Fig. 1; McNabb et al., 2008). The detection was based on positive mouse bioassays (MBA), the regulatory method at that time, and the toxin was eventually pinpointed as pinnatoxin (PnTX). Pinnatoxin was also detected in archived Pacific oysters that had been collected in Northland and frozen between 1994 and 1999 following closures for oyster harvesting, again based on positive MBAs. The causative organism was determined in 2009 as *Vulcanodinium rugosum* (Nézan and Chomérat, 2011), a benthic, peridinoid dinoflagellate which occurred primarily in a non-motile form in sediments. The Aotearoa New Zealand isolates produced PnTXs E and F (Rhodes et al., 2010a, 2010b, 2011, 2012). No illnesses have been associated with *V. rugosum* in Aotearoa New Zealand (McNabb et al., 2012), but toxicity studies carried out both on mice (acute and gavage administration as well as feeding trials; Munday et al., 2012, 2014; Smith et al., 2014) and on rats (both hippocampal brain slices and canulation) demonstrated that PnTXs are highly potent and act as antagonists of muscle-type nicotinic acetylcholine receptors at the neuro-muscular junction (Hellyer et al., 2011, 2013, 2014, 2015). A link between a *V. rugosum* bloom and acute dermal irritation in swimmers has been reported in Cuba (Moreira-González et al., 2021).

Prorocentrum species were regularly found in benthic samples and *P. lima* was found to form extensive mats on sediments in Rangaunu Harbour (Rhodes and Syhre, 1995) and on seagrass (*Zostera capricorni*; Table 2; MacKenzie et al., 2011). The benthic dinoflagellate has been found on oyster farm infrastructure and growing epiphytically on seaweeds as well as being reported in the plankton, the latter mainly after storm disturbance of sediments. Isolates from Northland were prolific producers of okadaic acid (OA) with approximately 50% being present in ester forms, mainly as dinophysistoxin (DTX)-4 type sulphated esters. Dinophysistoxin-1 was usually present but at trace concentrations (Rhodes et al., 2001, 2006a). An unusual compound produced by *P. lima* from Rangaunu Bay was rangiputamide. An extract was non-toxic to mice (i.p. injection) and not appreciably cytotoxic to Vero or P388 mammalian cells. The physiological or potential ecological function of rangiputamide remains unknown (Miles et al., 2012).

Samples were collected in February 2014 from three locations in the Bay of Islands, namely Oke Bay, Te Uenga Bay and Motuarohia Island (Fig. 1). The dominant epiphytic dinoflagellate species identified by DNA sequencing and HTS (metabarcoding) were *A. thermaeum, A. massartii, Biecheleria cincta, C. malayensis, Ostreopsis* cf. *siamensis* (currently referred to as *Ostreopsis* sp. 9)*, P. lima, P.* cf. *maculosum, P. micans, P. rhathymum* and *P. triestinum,* but many other species were identified at that time (Smith et al., 2017b).

Fukuyoa paulensis has been identified on many occasions in Bay of Islands samples, identified by electron microscopy as well as by real-time polymerase chain reaction (PCR) assays and HTS metabarcoding. Morphological surveys have also identified this epiphytic species, always at low cell densities (Rhodes et al., 2014c; Smith et al., 2017a; Rhodes and Smith, 2019). *Fukuyoa* is a genus very closely related *Gambierdiscus*, which contains CTX producing species (the species identified in NZ was originally named *Gambierdiscus* cf. *yasumotoi*) and is usually found on macroalgae, particularly the coralline rhodophyte

Table 2

Selected epiphytic and benthic dinoflagellates isolated from macroalgae and sediments at sites in Northland, Aotearoa New Zealand, during sampling trips carried out from January 1994 to November 2021. Isolates from November 2021 expedition in bold.

Species	CICCM CAW code	GenBank code	Substrate	Isolation site	Toxins	Sampling trips
Amphidinium thermaeum	D375, D376	NR	Brown macroalgae	Marsden Cove	NT	Jul 2021
Coolia malayensis	D39	U92258	Mixed sea wrack	Ninety Mile Beach	Neg. MBA	Feb 1995
	D43	NR	Mixed macroalgae	Parengarenga	NT	Sept 1997
	D77	KJ422852 KR605283	″ ″	Rangiputa	Pos. MBA	Jan 1998
	D154	KR605285 KJ422855	″ ″	Rangiputa	NT	2008
	D175	KR605286 KJ422856	″ ″	Rangaunu Harbour	NT	2009
	D214	MW177910	″ ″	Bay of Islands	NT	Feb 2013
Fukuyoa paulensis	D210	NR	″ ″	Parekura Bay	NT	?
	D238	NR	″ ″	Te Uenga	44-MG	2013
	D306, D308	MN305995	″ ″	Te Uenga	44-MG	Feb 2019
Ostreopsis cf. *siamensis*[!]	NR	NR	″ ″	Rangaunu and Parengarenga Harbours	PLTX-like	Jan 1997
	D73–D75	NR	″ ″	Whatawhiwhi, Rangiputa	PLTX-like	Jan 1998
	D96	AB674878 AB674915	″ ″	KeriKeri	PLTX-like	1999
	D147	AB674879	″ ″	Mahinepuha	PLTX-like	Apr 2006
	D206	KJ422860	″ ″	Tapeka Point	PLTX-like	Feb 2013
	D372	NR	″ ″	Marsden Cove	PLTX-like	Jan 2021
	NR	**NR**	″ ″	**Te Rawhiti Point and Te Uenga Bay**	PLTX-like	**Nov 2021**
P. lima	NR	NR	Sediments	Rangaunu Bay and Bay of Islands	OA, DTX-1 (trace)	1994
	NR	NR	*Zostera capricorni*	Rangaunu Harbour	OA	Feb 2009
	D339	NR	*Corallina* sp.	Paihia	OA, DTX-1	Feb 2019
	D340, D354	NR	*Hormosira banksii,* Sargassaceae sp.	Te Uenga	OA, DTX-1	Feb 2019
P. malayense[†]	D158	NR	Mixed sea wrack	Ninety Mile Beach	Neg. OA, DTX-1	1994
P. rhathymum	D226	NR	Coralline turfs	Te Uenga	NT	2014
Vulcanodinium rugosum	D163, D166–168, D170, D171, D178	JF267773 JF267781	Sediments and attached to mangroves	Rangaunu Harbour	PnTX E, F	Feb 2009
	D296	NR	Sediments	″ ″	PnTX E, F	Feb 2010

CICCM: Cawthron Institute Culture Collection of Microalgae.

[#]: initially published as *C. monotis** (Rhodes and Smith, 2019); !: currently known as *Ostreopsis* sp. 9; †: assigned as *P. maculosum* in Rhodes and Smith, 2019.

[§]: identified from the common PLTX-like amine oxidation fragment; MBA: mouse bioassay; 44-MG: 44-methylgambierone; OA: okadaic acid; PLTx: palytoxin equivalents; PnTx: pinnatoxins; NT: not tested; NR: not registered.

Jania sp., in the Bay of Islands area. *Fukuyoa paulensis* does not produce CTXs or MTXs but does produce 44-MG, a compound also produced by all currently tested *Gambierdiscus* species and some *Coolia* and *Fukuyoa* species (Murray et al., 2021).

Blooms of the dinoflagellate genus *Ostreopsis* have been thoroughly investigated in Aotearoa New Zealand and the species *O.* cf. *siamensis* (=*Ostreopsis* sp. 9) forms extensive blooms in the austral summer most years (Rhodes et al., 2000; Rhodes et al., 2001; Shears and Ross, 2009). It is predominantly epiphytic or benthic and is rarely observed in regular phytoplankton monitoring samples collected from the water column (records from New Zealand's National Marine Biotoxin monitoring programme), although it has been recorded in seawater samples from more southern temperate waters. Even when blooms are visible, cells are not always detected in water samples as *O.* cf. *siamensis* usually occurs as mats attached to shallow reefs and macroalgae (Shears and Ross, 2009).

Although the main risk from PLTX-like compounds is eye and dermal irritation and inhalation, rather than ingestion, there is a concern for human health if *O.* cf. *siamensis*, which produces PLTX-like compounds, was ingested through consumption of shellfish contaminated with 'PLTX'. Studies to date suggest there is only a small likelihood of this occurring in Aotearoa New Zealand as when the toxin has been detected in shellfish gut contents the concentrations have been low (Briggs et al., 1998; Rhodes et al., 2002). Crabs may take up PLTX-like compounds through feeding on contaminated shellfish (Rhodes et al., 2008) and kina (sea urchins; *Evechinus chloroticus*) are particularly vulnerable to *O.* cf. *siamensis* blooms in New Zealand as mass mortalities have occurred (Shears and Ross, 2009). A kina developmental assay was sensitive to the presence of PLTX-containing *O.* cf. *siamensis* extracts (Argyle et al., 2016).

In Aotearoa New Zealand's far north, three quarters of people surveyed regarding their shellfish consumption rates in 2021 confirmed that shellfish were consumed regularly, with most being harvested recreationally rather than commercially. Uptake of toxins by shellfish is thus a critical risk to this community (Guy et al., 2021). Phytoplankton monitoring is carried out regularly, but this does not give a true indication of the benthic and epiphytic microflora of the region. The results of a sampling trip to Northland in November 2021, which was focused on epiphytic dinoflagellates, are presented in this paper. There is a concern that with increasing sea water temperatures toxic benthic and epiphytic blooms (BHABs) may increase with resultant illnesses and economic stress in Northland (Fig. 2).

2. Materials and methods

2.1. Sampling, isolation and culture

Sampling at Rangitāhua was carried out on 26 November 2021 during the first scientific voyage as part of the New Zealand Ministry of Business, Innovation and Employment (MBIE) Endeavour Programme, Te Mana o Rangitāhua, led by Ngāti Kurī and Auckland War Memorial Museum. The multi-disciplinary team included Auckland Museum, the National Institute of Water and Atmospheric Research (NIWA), the University of Auckland, Massey University, and Manaaki Whenua. The voyage to Rangitāhua was on the superyachts MY Dapple and The Beast, which was made possible by philanthropic support.

Sampling was carried out by divers at Denham Bay, Raoul Island, (latitude/longitude: 29.285°S, 177.9536°W) at depths of 8–16 m (Fig. 1). Macroalgae (Table 1) were shaken into containers of local seawater to dislodge dinoflagellates and then removed. Sub-samples of the seawater were then transferred to 50 ml tubes (Corning CentriStar, Shanghai, China) containing germanium dioxide and f/2 medium (1% final conc. each; Guillard, 1975). As well as macroalgae being shaken into tubes of seawater, extra samples were also collected for analysis by HTS metabarcoding as described in Smith et al. (2017a).

Samples were couriered to Cawthron Institute on arrival of the ship in New Zealand and dinoflagellate cells were isolated (1 December 2021) using modified pickers and transferred to 12-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ, USA) containing f/2 medium and filtered seawater (ratio of 1:3). An exception were the *Prorocentrum* isolates which were cultured in metals mix SWii (Matsuda et al., 1996; Nishimura et al., 2020) or K media (Keller et al., 1987). All isolates were grown at 25 °C (± 2 °C); 40–70 μmol m^{-2} s^{-1} photon irradiance; 12:12 h L:D. Selected clonal cultures are currently maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM; Table 1).

DNA from dinoflagellate cultures was extracted and the D1-D3 region of the large subunit ribosomal RNA gene (LSU rDNA) was amplified and sequenced as described in Rhodes et al. (2017b) using primers D1R-F and D3B-R. The sequences were compared to existing sequences in GenBank using the BLAST online software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) as described previously (Rhodes et al., 2014a).

Sampling in the Bay of Islands, Northland, Aotearoa New Zealand, was carried out as described above at Te Uenga Bay (latitude/longitude:

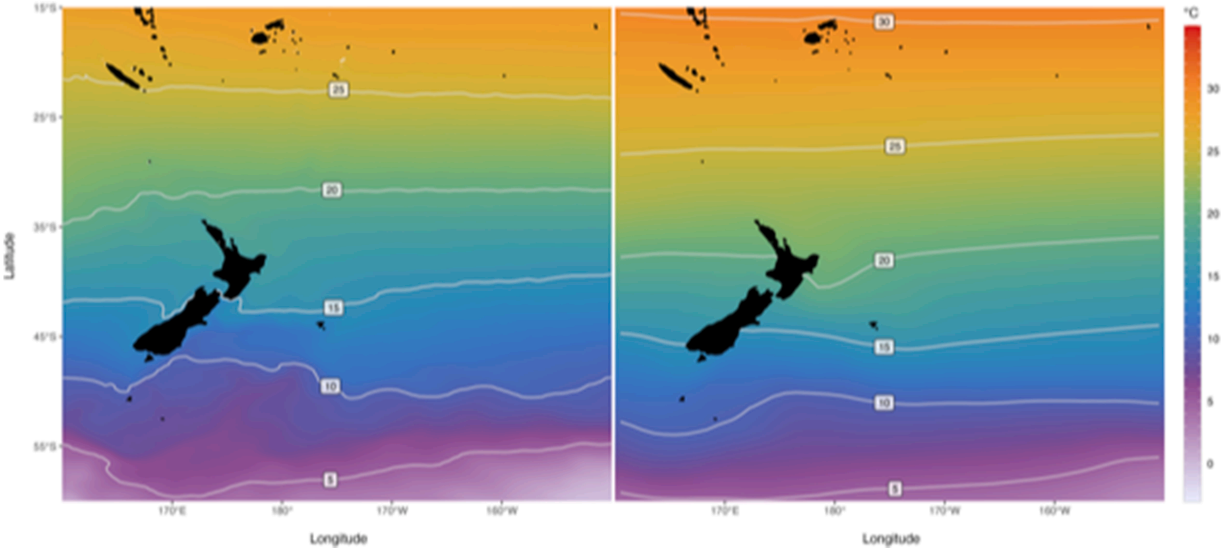

Fig. 2. Left: Average sea surface temperatures in the south-west Pacific from 1993 - 2020; Right: Projected average sea surface temperatures for 2070 - 2099. Data sources: Mercator Ocean's Global Reanalysis GLORYS 12v1 (https://www.mercator-ocean.eu/) and Scenario SSP 8.5 (IPCC 2021). Temperature isotherms (white lines) are shown for every 5 °C.

35.2558°S, 174.2417°E) on the 22 November and at Te Rawhiti Inlet (latitude/longitude: 35.2359°S, 174.2121°E), on 23 November 2021 (Fig. 1).

2.2. Toxin analyses

Cultures of *Gambierdiscus* (10 L), *Ostreopsis* (5 L), *Coolia* (2 L), and *Prorocentrum* (50 ml) were grown under the conditions outlined above until the cultures reached stationary phase. Cell counts (cells/ml) were carried out using Ütermohl counting chambers (10 ml of seawater sample or isolate in medium was added to chamber and settled for 4 h; the whole chamber was then counted), except for *Prorocentrum.* For *Prorocentrum,* 10 ml of media was removed from the bottom of the container using a micropipette and transferred into a 15 ml centrifuge tube (339,650; Thermo Fisher Scientific, MA, USA). Aliquots (100 µl) of each culture were transferred into a 1.5 ml screw-cap microcentrifuge tube (Labcon, California, USA) containing 890 µl of sterile seawater and 10 µl of Lugol's iodine solution (final concentration 1% (v/v)). The cell counts were then conducted in triplicate drops on a boundary slide glass (S6113; Matsunami Glass, Osaka, Japan) using an inverted microscope (CK-2; Olympus, Tokyo, Japan). For both methods the total volume was recorded. Cultures were then harvested by centrifugation (3200 × *g*, 15 °C, 15 min), the growth media decanted and cell pellets stored in the freezer (–20 °C) until extraction.

The *Gambierdiscus, Coolia* and *Prorocentrum* cell pellets were extracted, sonication aided (59 Hz for 10 min), using either a double- or single-stage procedure (for *Prorocentrum*) with 90% aqueous methanol (aq. MeOH). The *Ostreopsis* isolates were extracted using a two-stage procedure, sonication aided (59 Hz for 10 min), with 50% aq. MeOH + 0.1% acetic acid. All extracts had a final concentration of approximately 200,000 cells/ml.

Extracts were analysed using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) methods, with the microalgal species determining the toxin class(es) investigated and the individual analogues selected based on what reference material was available at Cawthron (either generated in-house or donated by research collaborators): *Gambierdiscus* and *Coolia* for P-CTXs (LoD 0.01 pg/cell; P-CTX-3B, P-CTX-3C, isoP-CTX-3B/C, P-CTX-4A, P-CTX-4B, isoP-CTX-4A/B; Murray et al., 2019), maitotoxins (MTXs; Detected/Not detected; MTX-1, MTX-5, MTX-6 and MTX-7; Murray et al., 2022), gambierone and 44-MG (LoD 0.01 pg/cell; Murray et al., 2021); *Ostreopsis* for PLTX-like compounds (PLTX, isobaric PLTX, ovatoxin (OVTX) a–e and ostreocin (OST) A/B and OST-D) using both intact (LoD 0.03 pg/cell; Murray et al., unpublished, refer Fig. 3) and oxidative cleavage methods (LoD 0.01 pg/cell; Selwood et al., 2012) and the *Prorocentrum* isolates for okadaic acid (OA), dinophysistoxin (DTX)-1 and DTX-2 (LoD 0.03 pg/cell; McNabb et al., 2005). LC-MS/MS analysis was carried out on a Waters Acquity UPLC i-Class system (Waters, Milford, MA, USA) coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer equiped with electrospray ionization (ESI; Waters, Manchester, UK). The reference material outlined above was used to generate multiple reaction monitoring transitions, quantitiation and confirmation, for the 25 hydrophilic and lipophilic toxins. Chromatographic seperation was achieved using four column-mobile phase combinations: CTXs, MTXs and gambierones were seperated using a Waters BEH Phenyl column (1.7 µm, 100 × 2.1 mm) that was eluted with ammoniated (0.2% NH_4OH, *v/v*) mobile phases (A) Milli-Q water and (B) acetonitrile (MeCN; Murray et al., 2019); seperation of the PLTX-like compounds for the intact method used a Waters BEH C_{18} column (1.7 µm, 50 × 1.0 mm; Murray et al., unpublished; Fig X) and for the oxidative cleavage method a Waters HSS C_{18} column (1.7 µm, 50 × 1.0 mm; Selwood et al., 2012), both eluted with acidifed (0.1% formic acid, *v/v*) mobile phases (A) Milli-Q and (B) MeCN; and the OA and DTXs were seperated using a Waters BEH Shield RP18 column (1.7 µm, 50 × 2.1 mm) that was eluted with buffered (2.53 mM ammonium hydroxide + 50 mM formic acid) mobile phases in (A) 5% acetonitrile (*v/v*) and (B) 95% acentronitrile (*v/v*; McNabb et al., 2005). All solvents used were LC-MS grade.

Data acquisition and processing were performed with TargetLynx software (Waters-Micromass, Manchester, UK). The toxin analogues were identified in the microalgal extracts based on retention time and fragment ion ratios in comparison to the reference material (either calibrated standards or toxin-positive cultures). Where applicable, quantification was performed using four or five point linear regression calibration curves.

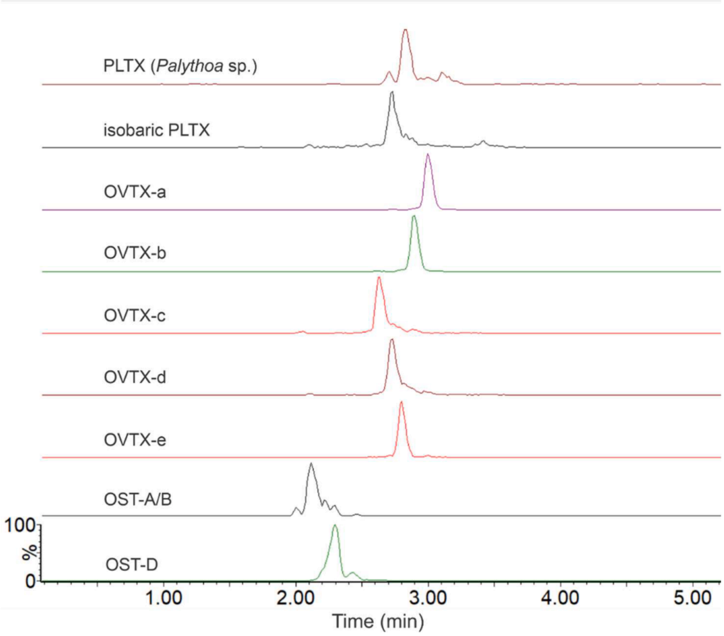

Fig. 3. Chromatogram showing the elution of the nine PLTX-like analogues monitored as the intact method (unpublished; PLTX: palytoxin; OVTX: ovatoxin; OST: ostreocin).

3. Results

3.1. Rangitāhua Kermadec Islands–expedition November 2021

The dinoflagellates collected during the most recent expedition to Raoul Island were from macroalgae sampled at Denham Bay, which stretches along the west coast of Raoul Island (Fig. 1). The macroalgae were growing on rocky reefs at depths of 8–12 m (Table 1; Fig. 4).

Many of the epiphytic dinoflagellate genera isolated have also been isolated on previous occasions and included species in the genera *Amphidinium, Coolia, Gambierdiscus, Ostreopsis* and *Prorocentrum* (Table 1). The microalgae were isolated from many different macroalgae, with no obvious substrate preference for the dinoflagellates.

Dinoflagellate species not previously identified in the region included *C. canariensis, C. palmyrensis,* and *C. tropicalis,* all identified by analysis of DNA sequencing data. *Coolia malayensis* was also re-isolated. *Coolia tropicalis* (CAWD384 and CAWD388) produced 44-MG (2.4 and 4.1 pg/cell, respectively) as did new isolates of *G. polynesiensis* (CAWD378 and CAWD380; 10 and 27 pg/cell, respectively) and *G. australes* (CAWD381; 127 pg/cell). *Gambierdiscus australes* (CAWD381) also produced MTX-1 and MTX-5 (qualitative, approximate ratio of 15:1). Both isolates of *G. polynesiensis* (CAWD378 and CAWD380) produced gambierone (54 and 84 pg/cell, respectively), and P-CTX-3C was also detected in the CAWD380 isolate (below the limit of reporting; Table 1).

Amphidinium carterae, A. massartii and *Ostreopsis tairoto* (previously *Ostreopsis* 3) were again isolated and identified by DNA sequencing (Table 1). *Ostreopsis tairoto* (CAWD383) was first analysed using the oxidative cleavage method (Selwood et al., 2012) and the common PLTX-like (PLTXs, OVTXs and OSTs) amine oxidation fragment was detected (0.08 pg/cell PLTX equivalence), yet none of the corresponding amide fragments. The isolate was then analysed for the nine PLTX-like analogues using the intact method, with none detected. Indicating an ‘unknown’ PLTX-like compound is produced by this *Ostreopsis* isolate (CAWD383).

Toxin producing *Prorocentrum* species isolated included *P. hoffmannianum* (OA and isoDTX-1) and *P. lima* (OA, DTX-1, and isoDTX-1). Isolates of *P.* cf. *emarginatum* and *P.* cf. *fukuyoi* did not produce toxins tested (Nishimura et al., unpublished).

Metabarcoding was carried out on thirteen samples collected from macroalgae in Denham Bay, Raoul Island. *Gambierdiscus* was detected in many of those samples (six out of 13 samples; Fig. 5) and was the dominant genus in some samples. *Gambierdiscus* reads matched with sequences from *G. polynesiensis* using BLAST (data not shown). Other potentially toxic genera detected included *Amphidinium, Coolia* and *Prorocentrum. Ostreopsis* was not detected on this occasion.

3.2. Northland, Aotearoa New Zealand–expedition Rawhiti November 2021

Seawater samples containing epiphytic dinoflagellates, isolated during the November 2021 sampling trip to Northland, were identified by analysis of DNA sequencing data. Unialgal cultures (10 L) of *O.* cf. *siamensis* (*Ostreopsis* sp. 9; $n = 4$), isolated from Rawhiti and Te Uenga, were grown and analysed for PLTX-like compounds. As above, the *Ostreopsis* isolates ($n = 4$) were first analysed using the oxidative cleavage method (Selwood et al., 2012), with the common PLTX-like amine fragment detected in all isolates (ranging from 0.02 to 0.08 pg/cell PLTX equivalence). The specific amide oxidation fragment corresponding to bishomoPLTX was also detected in all isolates (ranging from 0.01 to 0.03 pg/cell PLTX equivalence). No other specific amide oxidation fragment, corresponding to PLTXs, OVTXs or OSTs, were detected in any of the isolates. The four isolates were subsequently analysed for the PLTX-like analogues using the intact method, which included monitoring for the intact mass of bishomoPLTX. There were no detections in the isolates. This discrepancy is indicative that an unknown compound, modified between the amine and amide oxidation fragments compared to bishomoPLTX, is produced by these isolates.

A single strain of *Scrippsiella donghaiensis* was isolated and *A. carterae, A. operculatum* and *P. lima* were identified by light microscopy.

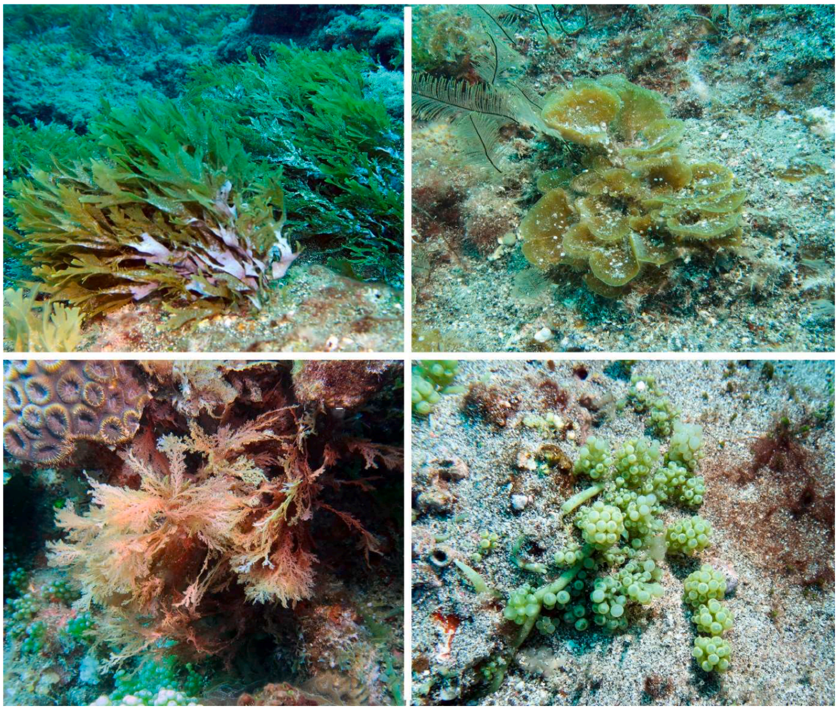

Fig. 4. Macroalgae from Rangitāhua Kermadec Islands which host epiphytic dinoflagellates. Top left: *Dictyota intermedia*, right: *Distromium* sp.; bottom left: *Plocamium* sp. and right: *Caulerpa racemosa.*

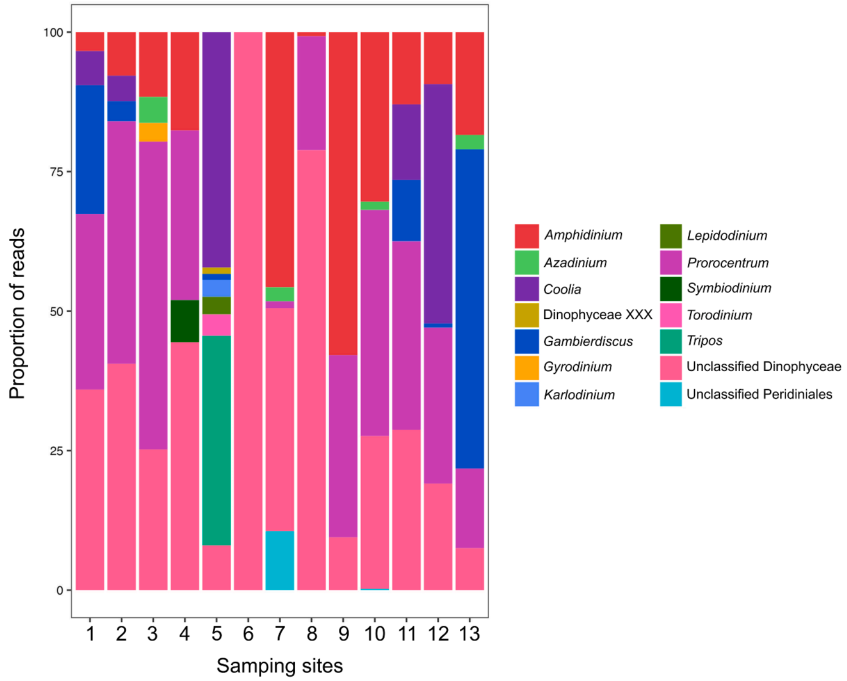

Fig. 5. Bar plots of the high throughput sequencing metabarcoding of samples from Denham Bay, Raoul Island, Rangitāhua Kermadec Islands. Plots show relative abundance of Dinophyceae reads at the genera level.

4. Discussion

Climate change is already being observed in Aotearoa New Zealand's sub-tropical north with coastal sea waters warming (Fig. 3; Ministry for the Environment, 2018; Sutton and Bowen, 2019; Dai et al., 2023). Other expected changes include increased rainfall in some regions and drought in other areas. The increased rainfall is likely to result in greater inputs of trace metals and organic nutrients into the coastal waters with potential impacts on bloom development and toxin production for some dinoflagellates (Rhodes et al., 2006b; Rhodes and Smith, 2022). New species may be introduced if the direction of major currents alters due to warming temperatures, as has happened to the East Australian Current (Australian Fisheries Management Authority, 2019), and this could include CTX-producing species of *Gambierdiscus* with the associated potential risk of ciguatera poisoning (CP). The uptake of MTXs by New Zealand snapper (*Pagrus auratus*) has already been demonstrated (Kohli et al., 2014) and Ledreux et al. (2014) have demonstrated the uptake of CTXs by herbivorous fish during *in vitro* experiments. If *G. polynesiensis* becomes established in Aotearoa New Zealand coastal waters, there is a real risk of CP occurring.

A single cell of *O. tairoto* was isolated from one live sample from Rangitāhua Kermadec Islands but was not detected by metabarcoding. It is possible this species is rare and was absent from the sample collected for metabarcoding as all other genera observed in the live samples, plus additional genera, were detected by metabarcoding, including *Gambierdiscus*.

Ostreopsis cf. *siamensis* is a regular bloom former in Aotearoa New Zealand and produces PLTX-like compounds. In addition, *O. ovata* and *O. lenticularis* have been recorded, based on morphological examinations, although rarely and in low concentrations (Chang et al., 2000). A serious potential human health issue is respiratory illness due to the inhalation of aerosols produced during *Ostreopsis* blooms, as has occurred in recent years in the Mediterranean. The key *Ostreopsis* species of concern there is *O. ovata*, a producer of isobaric PLTX and OVTXs (Ciminiello et al., 2006; Brescianini et al., 2006; Amzil et al., 2012). PLTX has not caused health issues in Aotearoa New Zealand to date, but as the coastal seas warm it is possible that *O. ovata* could become established as a dominant HAB species with accompanying health risks (Rhodes and Smith, 2022).

Vulcanodinium rugosum has been found forming thick mats on sediments in Rangaunu Harbour, only occasionally occurring in the water column in the motile life stage (Rhodes et al., 2011). Phytoplankton monitoring does not give an adequate risk warning of potential contamination of farmed or recreationally harvested shellfish by benthic species. A reliable and validated sampling method is the basic requirement for toxic benthic dinoflagellates and the artificial substrate method of Tester et al. (2014) would allow quantification. This substrate is lightweight, inert, and inexpensive with precisely quantifiable surface areas. One drawback is the need to leave the samplers out for 24 h, which entails two trips before samplers can be analysed, but once validated the method could be implemented in recreational shellfish harvesting areas as well as in CP risk areas. Combined with molecular (e.g., quantitative PCR and HTS) and chemical (e.g., SPATT bags) detection methods, monitoring and regulation will become a reality.

It is expected that new species of potentially harmful, benthic dinoflagellates will continue to be recorded in Aotearoa New Zealand and the research results from Rangitāhua are a guide to the species to expect in sub-tropical Northland as the oceans continue to warm.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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