The marine dinoflagellates *Heterocapsa horiguchii* and *Gambierdiscus polynesiensis* isolated from Norfolk Island

Fig. 1. Map of Norfolk Island showing proximity to Australia and Aotearoa New Zealand.

Norfolk Island is an Australian territory lying almost equidistant from Australia and Aotearoa New Zealand (1,600 km and1,200 km, respectively; Fig. 1). It is one of Australia's most isolated communities and oldest territories, having been settled six weeks after the founding of Sydney.

The Norfolk Island Marine Expedition in May 2025 (Fig. 2) was led by the Australian Museum [1] in collaboration with Tāmaki Paenga Hira Auckland War Memorial Museum, Te Papa Tongarewa the Museum of New Zealand, the University of Sydney, Parks Australia, and the Norfolk Island community. The aim was to monitor, protect and understand the biodiversity of the bioregion, including collection of seawater and macroalgae samples, as previously described [2]. Samples were couriered to the Cawthron Institute to determine the microalgae present. Preserved samples were used for DNA metabarcoding, and unpreserved samples allowed isolation and culture of dinoflagellates [3].

Fig. 2. Sample collection, Norfolk Island expedition 2025. Image courtesy of Tom Bannigan.

One isolate, collected from filmy red macroalgae at a site west of 'The Arch' on the north coast, was a small fast-moving dinoflagellate which multiplied rapidly in culture (Fig. 3; maintained in f2 medium at 25°C under standard light conditions [3]). The average cell length was 25.4 µm (n=10; range 21.4–28.1 µm) and average width 16.8 µm (n=10; range 14.5 µm–18.3 µm). DNA sequencing determined the isolate to be *Hetercapsa horiguchii* Iwataki, Takayama & Matsuoka, originally described from Japanese waters [4]. It has also been reported from Chinese waters and shown to be toxic in *Artemia salina* assays and haemolytic to rabbit erythrocytes [5], making it a potential harmful algal bloom (HAB) species. The Norfolk Island isolate is now maintained in the Cawthron Institute Culture Collection of Microalgae (CICCM), designated CAWD473 [6].

Hetercapsa horiguchii CAWD473 was tested for toxicity using mice by the intraperitoneal injection of culture extracts prepared according to the protocol of Munday et al. (2017) [7]. Single mice were dosed at 100, 250 and 750 mg/kg of culture extract (corresponding to 172,170,213; 430,425,531 and 1,291,276,596 cells/kg, respectively). Mice showed some initial signs of discomfort, such as abdominal stretching, but appeared normal within six hours post-dosing. In the first 24-hour period, post-dosing food intake was low, and the mice lost weight but for the following 13 days of the observational period mice gained weight with and showed normal food intake. At 14 days mice were killed, organ weights were all within normal limits. Since no toxicity was observed at any of the dose rates tested it can be concluded that the LD_{50} of *H. horiguchii* exceeds 750 mg/kg (1,291,276,596 cells/kg) making it as of low toxicity.

While *H. horiguchii* is a new record for Norfolk Island, other *Heterocapsa* species have been reported previously from Australian waters [8]. *Heterocapsa ovata* Iwatake & Fukuyo was isolated from Coffin Bay, South Australia, in 2014 and linked to fish and oyster deaths at that time. Isolates, bioassayed for ichthyotoxicity using cells from rainbow trout (*Oncorhynchus mykiss*) gills, were positive with the highest ichthyotoxicity observed in lysed cells. High

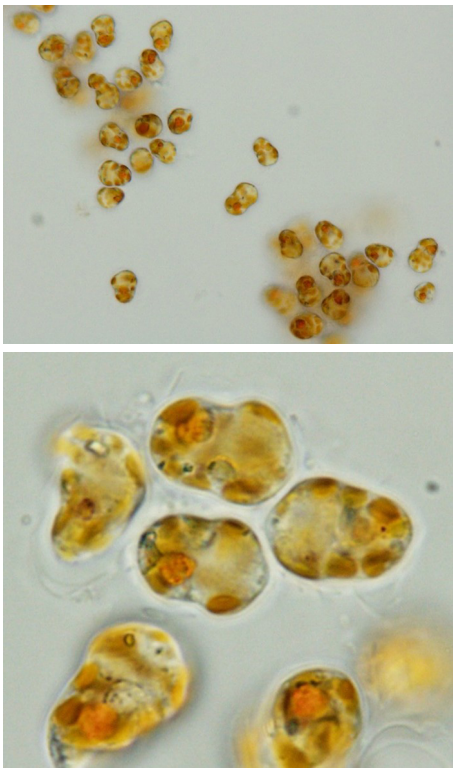

Fig. 3. Light micrographs of live Heterocapsa horiguchii *cells in culture: Top, mag. x80; Bottom, mag. x200, oil immersion (actual length 13.1 µm, width 9.6 µm).*

inter-strain variability suggested that different strains of *H. ovata* had varying levels of toxicity [8]. Other *Heterocapsa* species recorded in Australian waters include *H. niei* (Loeblich) Morrill & Loeblich, *H. triquetra* (Ehrenberg) Stein [9], and *H. rotundata* (Lohmann) Hansen [9-10].

Heterocapsa species previously reported in Aotearoa New Zealand include *H. niei*, *H. triquetra* [2,11] and *H. rotundata* [12] and, also *H. circularisquama* Horiguchi and *H. illdefina* (Herman & Sweeney) Morrill & Loeblich [11].

DNA sequencing determined the *Gambierdiscus* isolate to be *G. polynesiensis*, and toxin testing [13] indicated production of both 44-methylgambierone (5 pg/cell) and gambierone (4 pg/cell). An as yet unknown compound was also detected [14] and research into this compound is ongoing. Ciguatoxins (CTXs), the cause of the serious illness ciguatera poisoning, were not detected, but as the cell concentration was low it is possible that CTXs are produced at low or even trace levels. This will be investigated further once the extremely slow growing culture begins to thrive.

Samples preserved for metabarcoding were processed as previously described, targeting the large subunit ribosomal RNA gene region (D1-D2) [15]. The samples were filtered for reads identified as Dinophyceae and were dominated by reads identified as the epiphytic dinoflagellate *Ostreopsis* and the parasitic dinoflagellate genus *Amoebophrya* (Fig. 4). Other genera detected are mostly known to occupy planktonic habitats, including the genus *Heterocapsa* as reflected by the established culture. This reflects a low abundance of epiphytic/benthic taxa present in the samples. No sequence reads were identified as *Gambierdiscus* and potentially this taxon is rare and patchily distributed around Norfolk Island.

Given the proximity of Norfolk Island to northern Aotearoa New Zealand, it is only time before *H. horiguchii* and species of *Gambierdiscus* will be routinely detected in our coastal waters, and this could add a further risk to both the seafood industry and recreational fishing. They should therefore be added to those HAB species currently monitored in Aotearoa New Zealand.

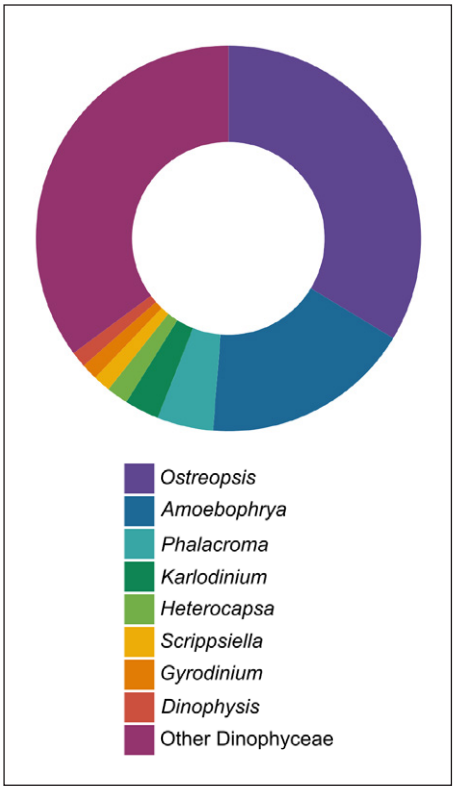

Fig. 4. Relative abundance of sequence reads identified as Dinophyceae using the large subunit ribosomal DNA region from samples collected around Norfolk Island. Genera that represent more than 1% of reads are shown.

Acknowledgements

Thanks to Sarah Challenger and Krystyna Ponikla (curators of the Cawthron Institute Culture Collection of Microalgae), Jacob Thomson-Laing and Veronica Beuzenberg for their assistance. Funding was provided by the NZ Ministry for Business, Innovation and Employment (Seafood Safety programme, Contract No. CAWX1801). Collections were conducted under Australian Marine Park Activity Permit PA2025-00023-1 issued 4 April 2025 by the Australian Government Director of Marine Parks.

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https://doi.org/10.5281/zenodo.17977076