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A fish kill associated with a bloom of *Amphidinium carterae* in a coastal lagoon in Sydney, Australia

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ABSTRACT

We report on a dense bloom ($\sim 1.80 \times 10^5$ cells mL^{-1}) of the marine dinoflagellate species *Amphidinium carterae* (Genotype 2) in a shallow, small intermittently open coastal lagoon in south eastern Australia. This bloom co-occurred with the deaths of >300 individuals of three different species of fish. The opening of the lagoon to the ocean, as well as localized high nutrient levels, preceded the observations of very high cell numbers. *A. carterae* is usually benthic and sediment-dwelling, but temporarily became abundant throughout the water column in this shallow (<2 m) sandy habitat. Histopathological results showed that the *Anguilla reinhardtii* individuals examined had damage to epithelial and gill epithelial cells. An analysis of the bloom water indicated the presence of a compound with a retention time and UV spectra similar to Luteophanol A, a compound known from a strain of *Amphidinium*. Assays with a fish gill cell line were conducted using a purified compound from cells concentrated from the bloom, and was found to cause a loss of 87% in cell viability in 6 h. The fish deaths were likely due to the low dissolved oxygen levels in the water and/or the presence of Luteophanol A-like compounds released during the bloom.

1. Introduction

Amphidinium species are amongst the most abundant benthic dinoflagellates in intertidal or estuarine marine sandy sediments in tropical, sub-tropical and temperate ecosystems (Murray and Patterson, 2002; Hoppenrath, 2000; Flø Jørgensen et al., 2004). Common species such as *Amphidinium carterae* Hulburt have a cosmopolitan distribution, occur worldwide, and grow readily and comparatively quickly. They are therefore commonly present in culture collections and have often been the subjects of physiological and genetic studies (i.e., Damjanovic et al., 2000; ten Lohuis and Miller, 1998).

Species of *Amphidinium* produce many different types of bioactive compounds (reviewed in Murray et al., 2012), with a wide range of toxicological impacts. Polyketides produced by *Amphidinium* species include macrolides, short linear polyketides, and long-chain polyketides. Amphidinols are one common type of polyketide compound produced by *Amphidinium carterae* and closely related species, with approximately 20 analogues known, displaying a variety of properties including haemolytic and antifungal activity (Satake et al., 1991; Paul et al., 1995, 1997; Morsy et al., 2005, 2006; Echigoya et al., 2005; Meng et al., 2010). A similar polyhydroxy compound, named luteophanol, was isolated from an uncharacterised species of *Amphidinium* inhabiting the acoel flatworm *Pseudaphanostoma luteocoloris* (Doi et al., 1997). The first polyketide synthase gene cluster sequenced from a dinoflagellate was from a strain of an unidentified species of *Amphidinium* (Kubota et al., 2006). Several unique and very divergent genotypes occur within the species *A. carterae* and the highly morphologically similar species *Amphidinium massartii*, including intraspecific variation of up to 38% in ITS rDNA sequences (Murray et al., 2004, 2012). Due to the presence of several cryptic species, molecular genetic sequencing is necessary for unequivocal identification of species and genotypes. Unfortunately, the vast majority of toxicological studies of species of

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Amphidinium have been conducted with unidentified strains (e.g., Tsuda et al., 2007).

Sudden mortalities of large numbers of fish and other marine life in coastal or estuarine habitats are common in south eastern Australia, with estimates of ~20 incidents annually reported to the Department of Primary Industries in New South Wales over the past 40 years, involving finfish, molluscs and crustaceans (NSW DPI, 2010). The causes of these fish kills are largely unexplored. In a small number of cases (~12%), chemical pollution or acid water inflow was detected and determined as the cause (Sammut et al., 1995; Roach, 1997). An extremely widespread fish kill in Jervis Bay, south eastern Australia in 2011 was due to a bloom of the toxic dinoflagellate *Karlodinium veneficum* (SM, unpub. data), a species that has previously caused fish kills in the region, in Lake Illawarra (Hallegraeff, 2002). In other Australian regions, fish kills have been attributed to blooms of the raphidophytes *Chattonella marina, Heterosigma akashiwo* or *Karenia* species (Hallegraeff, 2002). Generally, the causes of fish kills in Australian sites have not been fully investigated, although factors such as low dissolved oxygen levels, a change in salinity, temperature or pH were observed in some cases (~25% of cases) (NSW DPI, 2010).

Species of *Amphidinium* have very rarely been reported to be involved in pelagic harmful algal blooms (HABs) in shallow habitats (Lee et al., 2003; Sampayo, 1985; Gárate-Lizárraga, 2012). One reported bloom of *Amphidinium carterae* (Genotype 2) occurred in sedimentation ponds fed by effluent water from fish farming in Israel (Lee et al., 2003). The authors studied the physiological ecology of the *A. carterae* strain and found it to be eurytrophic, and able to tolerate a very wide range of salinities, temperatures, and pH, with a capacity for luxury consumption of nitrate and phosphate for several generations (Lee et al., 2003). Recurrent seasonal blooms of *A. carterae* in shallow fish ponds in Sado, Portugal have been reported to correlate with fish kills (Sampayo, 1985). The chemical structure of amphidinols (Meng et al., 2010) and luteophanols (Doi et al., 1997) are very similar to that of karlotoxins (Van Wagoner et al., 2008, 2010), which are produced by the unarmoured dinoflagellate *Karlodinium veneficum*, and can lead to the deaths of fish (Deeds et al., 2002, 2006; Kempton et al., 2002). It is possible that the fish kills observed in Portugal were related to the presence of compounds produced during the bloom.

In September 2012, a dense bloom of the species *Amphidinium carterae* was found in a coastal lagoon in Curl Curl on the northern side of Sydney, in south eastern Australia. This bloom occurred concurrently with the deaths of at least three different species of fish, bream (*Acanthopagrus australis*), sea mullet (*Mugil cephalus*) and longfin eels (*Anguilla reinhardtii*). In this study, we investigated the water quality conditions at the time of the bloom, the identity and abundance of the phytoplankton present, the histopathology of the fish, and the presence of polyhydroxyl compounds in the water, in order to determine the cause of the fish deaths.

2. Materials and methods

2.1. Sampling site

Curl Curl Lagoon, including Greendale Creek, foreshores, and the bed of the lagoon is Crown land, and Warringah Council is the appointed reserve trust manager. The land is managed according to a Plan of Management adopted by Council and Department of Lands under the Crown Lands Act 1989. Warringah Council provided permission for sampling for this study, and notified a range of offices of the bloom as part of its duty of care, in particular, the NSW Environment Protection Authority and the NSW Department of Primary Industries, Fisheries. Fish sampling was conducted under a Section 37 permit from NSW DPI Fisheries, and overseen by a representative from NSW Fisheries. No protected or endangered species of fish were collected during the sampling conducted for this study.

Intermittently open coastal lagoon (ICOLL) systems are common along the subtropical and warm temperate east coast of Australia (Haines et al., 2006). Curl Curl Lagoon is a small, shallow (<2 m depth) ICOLL system, with a sandy bottom sediment, approximately ~0.06 km^2 with a volume of ~48 mL (Fig. 1). Much of its catchment is urbanized. The bottom sediment of the lagoon is comprised of marine sand, east of Griffin Rd Bridge. To the west of the bridge, the central basin is a mud flat during periods where the entrance is open.

Within the lagoon, the opening and closing of its entrance is the dominant physical process (BMT WBM, 2011). The entrance is closed approximately 80% of the time by a sandy berm. During closure periods, rainfall, catchment runoff and groundwater contribute to rising water levels in the lagoon. This eventually leads to overspill and the natural opening of the entrance, approximately 12–16 times per year. During these periods, the lagoon becomes tidal and catchment runoff drains into the sea. Occasionally, the entrance has been artificially opened by local residents.

2.2. Phytoplankton sampling

In response to reports of water discolouration and fish deaths, triplicate 500 mL phytoplankton samples were taken on 17th September 2012 and fixed in Lugol's iodine solution. Samples were counted in a Sedgewick Rafter Cell. A live phytoplankton sample (200 mL) was also taken on the 20th September and observed within 6 h using an inverted compound light microscope (Nikon, Tokyo, Japan) and 100× magnification in order to verify species identification. Three additional live samples (4 L) were taken on 20th September for DNA extraction and chemical analysis.

2.3. Environmental variables

Samples to estimate the quantity of heavy metals, nutrients and other water quality variables were analysed. Nutrients were analysed at the Sydney Water Analytical Services Laboratory, according to American Public Health Association for the Examination of Water & Wastewater (APHA) reference methods, were: pH (APHA 4500-H^+), ammonia (APHA 4500-NH_3 H), oxidised Nitrogen (APHA 4500-NO_3 I), total nitrogen and phosphorus (APHA 4500-NP H), NH_3 (APHA 4500-NH_3 H), and NH_4 (method not accredited). The following heavy metals were analysed: total arsenic, total cadmium, total chromium, total copper, total lead, total mercury, total nickel, and total zinc according to the reference method USEPA 6020.

Physicochemical data was measured with a YSI 6920 V2 multiparametre water quality sonde, and data was viewed and stored on a YSI 650 MDS data logger. Climate data was sourced from the Australian Bureau of Meteorology (BOM) online database (Australian Bureau of Meteorology, 2013). Air temperature data for the period preceding the bloom were sourced from BOM Station 066196 (Sydney Harbour, Wedding Cake West). BOM records daily measurements of global solar exposure (total solar radiation) (MJ m^{-2}). Daily averages for September 2012 were downloaded from the BOM online archives, for the closest weather station to the lagoon Collaroy (Station No. 066126, ~3.7 km from lagoon). In order to gain an estimate of photosynthetically active radiation (PAR), 50% of the available daily solar radiation (Monteith, 1969) was used.

A water level buoy (Curl Curl Station Number: 213426) operated by Manly Hydraulics Laboratory was located near Griffin Road bridge (Fig. 1) and routinely recorded water levels at 15 min intervals.