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

Shauna A. Murray [1, 2*], Gurjeet Kohli [1, 2], Hazel Farrell [1, 2], Zoe B. Spiers[3], Allen Place[4], Juan José Dorantes-Aranda[5], Jason Ruszyck[6]

[1] Plant Functional Biology and Climate Change Cluster, University of Technology, Sydney, NSW, Australia

[2] Sydney Institute of Marine Sciences, Mosman, NSW, Australia

[3]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle NSW, Australia

[4]Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Sciences, Baltimore, Maryland, USA

[5]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia

[6]Warringah Council, Dee Why, NSW, Australia

Running title: *Amphidinum carterae* bloom in a coastal lagoon

*corresponding author, Shauna.Murray@uts.edu.au

Abstract

We report on a dense bloom (~1.80 x 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. *Amphidinium carterae* is usually benthic and sediment-dwelling, but temporarily became abundant throughout the water column in this shallow (< 2m) 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 hours. 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.

Keywords: *Amphidinium carterae*, harmful algal bloom, amphidinol, fish kill, lagoon, luteophanol

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 (ie Damjanovic et al., 2000; ten Lohius 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 *Amphidinium carterae* and the highly morphologically similar species *Amphidinium massartii*, including intraspecific variation of up to 38% in ITS rDNA sequences (Murray et al., 2004; Murray et al., 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 *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. The authors studied the physiological ecology of the *Amphidinium 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 *Amphidinium 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; Kempton et al., 2002; Deeds et al.,

2006). 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. Warringah Council notified a range of offices of the bloom as part of its duty of care, in particular, the NSW Environment Protection Authority. Warringah Council also notified the NSW Department of Primary Industries, Fisheries, of the incident and a fisheries officer assisted in the response and cleanup of the fish kill. 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 (Figure 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 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

Routine phytoplankton sampling was undertaken fortnightly at Curl Curl Lagoon during the warmer months of the year from November 2011 until May 2012. Replicate 500 mL water bottles were taken from approximately 20 cm depth and fixed in Lugol's iodine solution. 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 hours using an inverted compound light microscope (Nikon, Tokyo, Japan) and 100x 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-NH3 H), oxidised Nitrogen (APHA 4500-NO3 I), total nitrogen and phosphorus (APHA 4500-N PH), NH_3 (APHA 4500-NH3 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, total zinc according to the reference method USEPA 6020.

Physicochemical data was measured with a YSI 6920 V2 multiparameter 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) (MJm^{-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, approx. 3.7 km from lagoon). In order 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 (Figure 1) and routinely recorded water levels at 15 min intervals.

2.4 Fish Pathology

Dead bream (*Acanthopagrus australis*), sea mullet (*Mugil cephalus)* and longfin eels (*Anguilla reinhardtii*) were observed in the lagoon from the 29th August – 26th September. Five moribund eels were euthanized, immediately fixed in 10% neutral buffered formalin solution and kept cool on 20th September. Samples were sent via courier to the State Veterinary Diagnostic Laboratory at the Elizabeth Macarthur Agricultural Institute where they were trimmed, embedded in paraffin and stained with haematoxylin and eosin for histology.

2.5 DNA extraction, PCR and sequencing

An aliquot of the water sample from 20th September was centrifuged at 3000 rpm for 10 mins and the resulting pellet was extracted using a modified CTAB method (Doyle and Doyle 1990). To amplify the internal transcribed spacer (ITS) region of the ribosomal RNA gene, the primers ITSfow (TTCCGTAGGTGAACCTGCGG) and ITSrev (ATATGCTTAAATTCAGCGGGT) were used (Murray et al 2012). PCR reactions contained template, 0.5 µM of each primer, 3 mM $MgCl_2$, 1 µL BSA (NEB), and 10 µL Immomix (Bioline, Taunton, MA), containing dNTPs, Immolase Taq polymerase and reaction buffer. Hot start PCRs were performed with an initial denaturing step of 95°C for 5-10 min, and 35 cycles of 30 s at 95°C, 30 s at 60-65°C, 30s at 72ºC followed by a final extension step of 7 min at 72°C.

Samples for DNA sequencing were prepared by adding 10mM of primer to at least 400 ng of purified DNA. Samples were prepared in 20 µL reactions of 1 µL Big Dye Terminator Mix (Applied Biosystems, Carlsbad, CA) and the sequencing reaction was run using the following PCR program: 96°C 3 min, 96°C 10 sec, 50°C 5 sec 60°C 4 min for 30 cycles. The sequences were assembled using the software Geneious (Kearse et al 2012). The sequences were submitted to GenBank (Accession Number KF356164).

The final alignment of 22 sequences of 434 nucleotides was initially aligned using MAFFT v6.814b (Katoh et al 2002) and manually inspected and checked. The program FindModel (Posada and Crandall 1998) was used to test and determine optimal model-fitting of the sequence data. The PhyML 3.0 algorithm (Guindon and Gascuel 2003) was used for Maximum Likelihood inference based on the optimal substitution model, the General Time-Reversible model with a gamma distribution. Five hundred bootstrap replicates were performed.

2.6 Chemical analysis

A 2 L sample of the discoloured water in the lagoon was taken on 17th September. The sample which contained 95,000 cells mL^{-1} was concentrated to a final volume of 4 mL by centrifugation (1000 rpm for 5 min) and then freeze dried. The cell pellet was extracted with 30% methanol in a sonication bath (20 mL) for 40 mins, diluted to 200 mL with HPLC water and loaded on 3 mL packed volume C-18 solid phase extraction cartridge column (Waters Corporation, Massachusetts USA). After the extract was loaded onto the column it was washed with 12 mL of increasing concentrations of methanol / water from 0% methanol to 100% methanol in 20% increments. All fractions were collected, dried under a vacuum, resuspended in 1 mL of methanol, and filtered with a GF/B filter prior to HPLC-MS analysis. The eluates were injected onto a C8 (LiChrosphere 125 mm x 4mm 5 μm bead size RP-8, Agilent) column and subjected to a 1 mL min^{-1} 10% to 95% methanol : water gradient over 25 min. using an Agilent 1100 HPLC. Toxin peaks were detected using an Agilent Diode Array Detector (Model#G1315B) with a micro high-pressure flow cell (G1315B#020; 6 mm pathlength, 1. 7 ul volume) over the wavelength range 190 to 950 nm. The entire UV spectra were saved for each UV detectable peak. The eluate from the DAD detector was mixed with 10 mM ammonium formate in methanol prior to introduction to the electro-spray nozzle of the MS (Agilent G1956A SL) for ionization with the following spray chamber conditions using nitrogen as the drying gas: flow rate 10 L min^{-1}. pressure 60 psi, temperature 350°C, fragmentor voltage 350 V, capillary voltage 3000 V. The 10 mM ammonium formate in methanol (0.1 mL min^{-1}) ensured appropriate pH conditions for negative mode ionization. The majority of the material was found in the 80% methanol fraction and was subsequently purified on a semi-preparative C18 column (9.4 cm x 590 mmm 5μm Zorbax) using a 30% to70% acetonitrile/water linear gradient.

2.7 Fish gill cell assay

The epithelial fish gill cell line RTgill-W1 was obtained from the American Type Culture Collection (CRL-2523) (Bols, 1994). This cell line was routinely cultured on 25 cm^2 culture treated flasks (690170,

Greiner Bio-One) with Leibovitz's medium (L1518, Sigma) supplemented with 10% fetal bovine serum (v/v) (12003C, Sigma) and an antibiotic-antimycotic solution (A5955, Sigma). Cells were incubated at 19ºC in the dark. Confluent gill cells were detached with 0.25% trypsin-0.02% EDTA solution (59428C, Sigma) for subculturing and seeding purposes as described by Dorantes-Aranda et al., (2011).

Gill cells were detached, counted and seeded at a concentration of 2×10^5 cells mL^{-1} in quadruplicate in a 96-well microplate (655180, Greiner Bio-One) 48 hrs prior to the experiments (Dorantes-Aranda et al., 2011). An extract purified from the bloom of *Amphidinium carterae* was dissolved in 30% methanol and mixed with L-15/ex medium (Schirmer et al., 1997) for the exposure (final methanol concentration was 6.7%). Gill cells were exposed to the extract at 0.1, 1, 10, 50 and 100 µg mL-1 for 2 and 6 hrs. After completion of this time period, experimental solutions were discarded and gill cells were rinsed with phosphate buffer saline (PBS). Control gill cells were exposed to L15/ex with methanol at 6.7%; preliminary experiments showed that 6.7% methanol does not have any effect on gill cell viability at 6 hrs.

Alamar blue (DAL1025, Invitrogen) in L-15-ex (5% v/v) was added to all wells and incubated for 2 hrs in the dark (Dayeh et al 2005) to measure viability of gill cells. Fluorescence of metabolised alamar blue was measured with a microplate reader (FLUOstar OPTIMA, BMG Labtech, 413-3350), using excitation and emission filters of 540 and 590 nm, respectively.

3. Results

3.1 Water quality

The majority of the water samples from 17th September were found to have heavy metal concentrations below or just above detection limits. They were well below ANZECC (2000) guidelines for ecosystem health. Values of total nitrogen (TN- sum of all dissolved, particulate, organic and inorganic nitrogen)

were 2.7 – 7.75 mg L^{-1} ± 0.46 (193-250 µM N), while values of total phosphorus (TP) were 0.198 – 0.310 mg L^{-1} ± 0.058 (6-23 µM P) (Figure 2). These values exceed ANZECC trigger levels for TN (0.12 mg L^{-1} (marine) and 0.30 mg L^{-1} (estuaries)) and TP (0.025 mg L^{-1} (marine) and 0.03 mg L^{-1} (estuaries)). While there is no criteria within the guidelines for TN:TP ratio and eutrophication, these values are over an order of magnitude higher than what would be considered to be a "slightly disturbed ecosystem" (ANZECC 2000). The molar ratio of TN to TP was approximately 26:1 over the three sampling stations. This was greater than the nominal Redfield ratio of 16:1 and suggested that phosphorus was limited within the lagoon. Levels of oxidised nitrogen ($N0_2$- and $N0_3$-) and ammonia (NH^{4+} and NH^3) were below detectable limits (<0.01 mg L^{-1}). Dissolved oxygen levels on the 18th September were low, 10.5% ± 0.2 (0.91 mg L^{-1} ± 0.02). Temperature was 18.5 °C ± 0.47 and salinity was 12.6 ‰ ± 2.44 on the 18th September. pH was 9.0-9.4 ± 0.06 on the 18th September.

3.2 Environmental variables

On August 28th 2012, the mouth of the lagoon was illegally opened, causing it to drain to the sea. In the lead up to, and during the bloom event, water level data recorded by MHL (Griffin Rd data buoy) in the lagoon showed a gradual increase from 1.2 m on 10 September to 1.7 m on 30 September, but it had not reached the natural spill level of ca. 2 m. Depending on light conditions within the water column (Parslow et al 1999), PAR could range between 78 and 475 µE m^{-2} s^{-1} within a 2 m water column, based on 50% of the available solar radiation (18 MJm^{-2}). Rainfall data from the Collaroy gauge was 0 mm in the weeks preceding the bloom, indicating that sudden changes in salinity are unlikely to have occurred related to rainfall. Daily air temperature was 16.0 – 26.7 °C in the two weeks preceding 15th September (Sydney Harbour, Wedding Cake West). These temperatures are within the normal range experienced in September in this area. Based on the local weather conditions, water column turbulence was assumed to be low within the lagoon.

3.3 Phytoplankton abundance

Amphidinium carterae was not observed in routine monitoring samples collected in the lagoon between November 2011 and April 2012. The phytoplankton samples taken from the bloom on 17th September 2012 were found to be dominated by the single species *Amphidinium carterae*, which showed an abundance of 36-186 x 10^3 cells mL^{-1} (Figure 2). During the bloom the water was a yellow-brown colour. No distinct odours were reported. In the period following the bloom, it appeared that the cells of *Amphidinium* had sunk and settled on the bottom of the lagoon.

3.4 Identification of the species

As several species of *Amphidinium* are morphologically highly similar, and as three distinct genotypes of *Amphidinium carterae* exist, we conducted a detailed morphological examination of the samples and molecular genetic sequencing studies. Cells of *Amphidinium carterae* were approximately oval, 8-12 µm long (n=20), with a very small, left deflected epicone and a yellow-brown plastid, which filled the cell.

In order to determine which genotype of *Amphidinium carterae* was present, we amplified and sequenced the ITS rRNA region directly from DNA from a water sample from the lagoon taken on 20th September, using PCR primers that amplify those of any eukaryote. A sole clean sequence was recovered, and was found to be highly similar (99.5%) to Genotype 2 of *Amphidinium carterae*. Phylogenetic analysis was performed, and sequences belonging to *A. carterae* formed a clear monophyletic group, with three well-supported clades representing three different genotypes of *A. carterae* (Figure 3), which is in accordance with previous studies (Murray et al 2012). The query sequence clustered with other reference sequences belonging to Genotype 2 of *A. carterae*, further confirming the identity of the strain (Figure 3).

3.5 Fish Pathology

Approximately 300 dead fish were found and removed from the lagoon from 17^{th} – 26^{th} September. These consisted of ~50% sea mullet (*Mugil cephalus)*, 30% bream (*Acanthopagrus australis*) and 20% longfin eels (*Anguilla reinhardtii*). Five individuals of *Anguilla reinhardtii* were submitted for histopathology examination. All eels displayed similar changes in varying severities. Consistent findings on histology included diffuse, sometimes marked epithelial hypertrophy, associated with epithelial hyperplasia, lamellar fusion, and mild infiltrates of predominantly lymphocytes. Other findings included a mild increase in mitoses of gill epithelial cells, individual cell degeneration and necrosis, surface bacterial proliferation and accumulates of cellular debris between lamellae.

3.6 Chemical analysis

The results of the chemical analysis on the extracted material showed the presence of a single abundant compound (Figure 4, lower panel), with retention time and UV spectra (Figure 4, upper panel) consistent with a luteophanol-A like compound, and not an amphidinol. The UV spectra indicated the presence of conjugated diene chromophore (Lambda max 232 nm) not a conjugated triene chromophore (Lambda max 280 nm) expected for amphidinol. The negative electronspray ionization mass spectra for the peak showed a quasi-molecular ion peak at m/z 1471.4 .$(M-H)^-$ and 1487.3 $(M-H)^-$(Figure 5). The presence of a sulfate ester was suggested by intense fragment ions observed [m/z 120 ($NaHSO_4^-$) and 103 ($NaSO_3^-$)].

3.7 Fish gill cell assay

Gill cells were sensitive to material purified from Curl Curl Lagoon bloom (Figure 6). Gill cells showed a decrease of viability of 27% after exposure to this compound at 0.1 - 50 µg mL^{-1} for 2 hrs. The same

effect was observed after 6 hrs of exposure but only at concentrations of 0.1, 1 and 10 µg mL^{-1} (no significant differences were observed). A major decrease in cell viability was observed at 50 µg mL^{-1} between 2 and 6 hrs (72.6 versus 36.4%). Gill cells were 48% (±2.7%) viable upon exposure to 100 µg mL-1 for 2 hrs, which went down to 13% (±4.5%) at 6 hrs. Median lethal concentration (LC50) was 95 and 23.5 µg mL-1 at 2 and 6 hrs, respectively (Figure 6).

4. Discussion

Phytoplankton samples collected during the Austral spring-summer period at Curl Curl Lagoon have been found to comprise a mixed assemblage of diatoms (*Thalassiosira, Navicula, Pseudo-nitzschia* spp.) with some presence of dinoflagellates (e.g. *Ceratium* spp., *Scrippsiella* spp., *Protoperidinium* spp.) (Murray, 2012). Species of the genus *Amphidinium* had not previously been identified during routine phytoplankton monitoring of the lagoon, which is not unexpected, as characteristically *Amphidinium* spp. are sand-dwelling rather than planktonic (Murray and Patterson, 2002). The observation of ~180 x 10^3 cells mL^{-1} (corresponding to a biovolume of 256 mm^3 l^{-1}) is substantially higher than the abundance of other species observed during the routine monitoring program, as other species were found to reach maximum cell concentrations of 10 cells $mL^{-1}$for individual species (e.g. *Prorocentrum minimum*).

At its most abundant, the bloom caused an extensive yellow-brown water discolouration in this shallow sandy lagoon system. This density of *Amphidinium carterae* is in the range of similar blooms of this species reported from Pakistan, Portugal and Mexico (Sampayo, 1985; Garate-Lizarraga, 2012, Baig et al., 2006). In the Sado estuary of Portugal, *A. carterae* has been found to occur in high abundances seasonally in fish ponds, corresponding with fish die-offs (Sampayo, 1985). Intertidal pools in the North Arabian Sea along the coast of Pakistan were reported to have blooms of *A.carterae* with concentrations of 12 × 10^3 cells mL^{-1} (Baig et al., 2006). In Mexico, densities of *A.carterae* were also high (28-64 × 10^3 cells mL^{-1}), however, no fish die-offs were observed (Garate-Lizarraga, 2012). A maximum growth rate

(μ_{max}) of 2.89 d^{-1} for *Amphidinium carterae* has been reported (Smayda, 1997). Similar findings of μ_{max} 2.7 d^{-1} have also been reported (Ismael et al 1999). Applying the formula $N_0 \times e^{\mu t}$ where N_0 was an initial cell concentration (10 cells mL^{-1}), cell concentrations of 180×10^3 cells mL^{-1} could be achieved within a very short time period (*t*) of 3-4 days.

Species of *Amphidinium* are typically found in marine shallow sediments, however some species are known to undergo vertical migration, and are occasionally found in the water column (Eaton and Simpson 1979). In the case of *A. carterae*, the species does not generally exhibit diel vertical migration, but has been reported to maintain its position within a 1 m range in the water column (Kamykowski and Zentara, 1977; Eggersdorf and Hader, 1991). Similarly, recurrent blooms in the Sado Estuary in Portugal were in water depths of ~0.8 m (Sampayo, 1985). This life strategy may be of benefit during optimal nutrient conditions, during which *Amphidinum carterae* may have the capacity for "luxury consumption" of nutrients and the ability to store reserves of both phosphorus and nitrogen (Lee et al., 2003). High pH levels during dinoflagellate blooms are not uncommon, and blooms of *Amphidinium* have been found to tolerate high pH levels (>8.5) (Lee et al., 2003; Hinga, 1992). In Curl Curl lagoon, the elevated pH levels may have been a result of fertilizer input or the uptake of carbon dioxide by the bloom during photosynthesis. Nutrient availability, light, salinity and temperature have all been demonstrated to affect toxin production by *Amphidinium carterae* (Zimmermann, 2006).

The analysis of the water samples showed that heavy metals were not present at high concentrations, and that pH was not in the range to indicate the presence of an acid inflow. A single abundant compound was found which had the same retention time and UV spectra as luteophanol A (Doi et al., 1997) (Figure 4). Luteophanol A is a polyhydroxy linear carbon chain compound that was first isolated from an uncharacterised species of *Amphidinium*, which had been isolated as symbiotic within a flatworm (Doi et al., 1997). Further investigation of this *Amphidinium* strain led to isolation of luteophanols B, C and D (Kubota et al., 1998; 2005). Polyhydroxyl compounds produced by dinoflagellates, such as amphidinols, have been reported to have haemolytic and antifungal activities (Echigoya et al 2005, Meng et al 2010).

Luteophanol D is the only luteophanol compound investigated to date for its biological activity, and it has been reported to have antibacterial effects (Kubota et al., 2005).

Exposure of fish gill cells to the bloom extract from *Amphidinium carterae* caused 27-35% decrease of cell viability at low concentrations (0.1-10 µg mL^{-1}), which increased with time and concentration (maximum of 87% at 100 µg mL^{-1}). The species *Amphidinium carterae* has high levels of intraspecific genetic variation, and consists of four known genotypes (Murray et al., 2004; Rhodes et al., 2010). Strains of two of these genotypes have been examined for their toxicological effects. A genotype two strain (strain CAWD 57) has shown haemolytic effects due to its production of amphidinols (Echigoya et al 2005). A raw extract of a genotype four strain was found to be highly toxic by mouse bioassay (Rhodes et al 2010), and an extract of a further uncharacterised genotype was found to have haemolytic activity (Nayak et al 1997). An uncharacterised strain of *Amphidinium carterae* was found to have impacts on sea urchin larval development, strong impacts on *Artemia salina* nauplii, and some haemolytic activity (Pagliara and Caroppo 2012).

A chemically closely related compound, karlotoxin, which belongs to a group of fish-killing toxins produced by *Karlodinium veneficum,* has been described (Van Wagoner et al., 2008). Karlotoxin KmTx 2 has proven to be toxic to fish gill cell cells as well; however KmTx 2 showed a higher cytotoxicity than this compound, with LC_{50} values ranging between 203-380 ng mL^{-1}, compared to LC_{50} = 23.5-95 µg mL^{-1} (Place et al., 2012). Karlotoxins may function by non-specifically increasing the ionic permeability of biological membranes, resulting in osmotic cell lysis and damage to gill epithelial tissues (Deeds et al., 2006).

The findings of the histopathological study of *Anguilla reinhardtii* collected at the site of the fish kill indicated epithelial hypertrophy, associated with epithelial hyperplasia, lamellar fusion, and a mild increase in mitoses of gill epithelial cells. Water quality analysis showed that the dissolved oxygen levels were very low, 10.5% ± 0.2 (0.91 mg L^{-1}) on the 18th September, two days prior to the collection

of the fish for histopathological analysis on the 20th September. Historical information is available on the dissolved oxygen levels at Curl Curl Lagoon over a 6 year time scale, taken at 9 sites within the lagoon (Supplementary Figure 1). This shows that levels are highly variable, and have been historically measured as occasionally as low as 0-6 mg L^{-1} at several sites over several years. There has been much research on the phenomenon of high biomass phytoplankton blooms leading to hypoxia in water bodies, due to the large respiratory oxygen demands of phytoplankton and accompanying bacterial communities (ie reviews are Diaz, 2001; Anderson et al., 2002; Diaz and Rosenberg, 2008). A study of dense blooms of *Prorocentrum minimum* in coastal embayments showed that the growth of *P. minimum* could reduce ambient dissolved oxygen levels of 10–12 mg L^{-1} to 0.1 mg L^{-1} within 4 days due to respiration demand in the dark (Brownlee et al., 2005). It has been shown that the growth of *Amphidinium* spp in culture was highest at a low ambient oxygen concentration of 5%, suggesting that its growth is not inhibited by low dissolved oxygen levels (Kitaya et al., 2008). Dense phytoplankton blooms leading to low dissolved levels have been frequently shown to result in fish kills (Diaz, 2001; Diaz and Rosenberg, 2008; Jones and Rhodes, 1994, and references therein).

It has been shown that *Anguilla* spp had 100% survival for 48 hours at very low dissolved oxygen levels of 1 mg L^{-1}, the level reported in this study, and other fish species showed survival rates from 20-85% (Dean and Richardson, 1999). For this reason we are cautious about attributing the cause of death of fish in the lagoon. Based on the observed gill damage, we suggest that it was likely due to the low dissolved oxygen levels, and the presence of the luteophanol-like compound may have contributed.

5. Conclusions

We report on a dense bloom (~180 x 10^3 cells mL^{-1}) of *Amphidinium carterae* (Genotype 2) in a shallow intermittently open coastal lagoon in south eastern Australia. The deaths of > 300 individuals of three different species of fin fish occurred concurrently. *Anguilla reinhardtii* individuals had damage to epithelial and gill epithelial cells. An analysis of the bloom water indicated the presence of a compound

with the retention time of a luteophanol A-, which caused up to 87% decrease in cell viability in an assay of fish gill cells. The fish deaths may have been attributed to the low dissolved oxygen, and the presence of a luteophanol A-like compound may have contributed.

Acknowledgements

We thank the Australian Research Council (grant DP120103199) and Warringah Council for funding. We thank Gustaaf Hallegraeff for helpful suggestions and finding literature. We thank Megan Burgoyne (Sydney Water) and Marcel Green (NSW DPI Fisheries) for technical assistance. This work was funded in part by grants from Oceans & Human Health NIH R01ES021949-01/NSF OCE1313888 (to R.Jagus and A.R.Place). This is contribution #4946 of the University of Maryland Center for Environmental Science, #14-131 of the Institute of Marine and Environmental Technology, and contribution #xxx from Sydney Institute of Marine Sciences.

References

Anderson DM, Glibert PM, Burkholder JM. 2002. Harmful algal blooms and eutrophication, nutrient sources, composition, and consequences. Estuaries 25, 704–726

Australian Bureau of Meteorology, http,//www.bom.gov.au/climate/data. Accessed 3/7/13

Agriculture and Resource Management Council of Australia and New Zealand. 2000. Australian and New Zealand guidelines for fresh and marine water quality. Volume 1, Australian and New Zealand Environment and Conservation Council.

Baig HS, Saifullah SM, Dar A. 2006. Occurrence and toxicity of *Amphidinium carterae* Hulburt in the North Arabian Sea. Harmful Algae 5, 133-140.

Bols NC, Barlian A, Chirino-Trejo M, Caldwell SJ, Goegan P, Lee LEJ. 1994. Development of a cell line from primary cultures of rainbow trout, *Oncorhynchus mykiis* (Walbaum), gills. Journal of Fish Disease 17, 601-611.

BMT WBM. 2011. Stormwater and estuary modelling for Curl Curl Lagoon, review of information. Report prepared for Warringah Council, New South Wales, Australia.

Brownlee EF, Sellner SG, Sellner KG 2005. *Prorocentrum minimum* blooms, potential impacts on dissolved oxygen and Chesapeake Bay oyster settlement and growth. Harmful Algae 4, 593-602.

Damjanovic A, Ritz T, Schulten K 2000. Excitation transfer in the peridinin-chlorophyll-protein of *Amphidinium carterae*. Biophysical Journal 79, 1695-1705.

Dayeh VR, Schirmer K, Lee LEJ, Bols NC 2005. Rainbow trout gill cell line microplate cytotoxicity test. In, Blaise C, Férard JF, editors. Small-scale freshwater toxicity investigations. The Netherlands, Springer. pp. 473-503.

Dean TL, Richardson J. 1999, Responses of seven species of native freshwater fish and a shrimp to low levels of dissolved oxygen. New Zealand Journal of Marine and Freshwater Research, 33,199-106

Deeds JR, Reimschuessel R, Place AR. 2006. Histopathological effects in fish exposed to the toxins from *Karlodinium micrum*. Journal of Aquatic Animal Health 18, 136–148.

Deeds JR, Terlizzi DE, Adolf JE, Stoecker DK, Place AR. 2002. Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae) –A dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful Algae 1, 169-189.

Diaz RJ 2001. Overview of hypoxia around the world. Journal of Environmental Quality, 30, 275-281

Diaz RJ, Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321, 926- 929

Doi Y, Ishibashi M, Nakamichi H, Kosaka T, Ishikawa T, Kobayashi J 1997. Luteophanol A, a new polyhydroxyl compound from symbiotic marine dinoflagellate *Amphidinium* sp. Journal of Organic Chemistry 62, 3820-3823.

Dorantes-Aranda JJ, Waite TD, Godrant A, Rose AL, Tovar CD, Woods GM, Hallegraeff GM 2011. Novel application of a fish gill cell line assay to assess ichthyotoxicity of harmful marine microalgae. Harmful Algae 10, 366-373.

Doyle JJ, Doyle JL 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

Eaton JW, Simpson P. 1979. Vertical migrations of the intertidal dinoflagellate *Amphidinium herdmaniae* Kofoid & Swezy, In, Naylor Eet al., editors.Cyclic Phenomena in Marine Plants and Animals, Proceedings of the 13th European Marine Biology Symposium, Isle of Man, 27 September-4 October 1978.pp. 339-345.

Echigoya R, Rhodes L, Oshima Y, Satake M. 2005. The structures of five new antifungal and hemolytic amphidinol analogs from Amphidinium carterae collected in New Zealand. Harmful Algae 4, 383-389.

Eggersdorfer B, Hader D 1991. Phototaxis, gravitaxis and vertical migrations in the marine dinoflagellates, *Peridinium faeroense* and *Amphidinium carterae*. Acta Protozoologica 30, 63-71.

Flø Jørgensen M, Murray S, Daugbjerg N 2004. *Amphidinium* revisited. I. Redefinition of *Amphidinium* Dinophyceae. based on cladistic and molecular phylogenetic analyses. Journal of Phycoly 40, 351-365.

Gárate-Lizárraga I 2012. Proliferation of *Amphidinium carterae* Gymnodiniales, Gymnodiniaceae. in Bahía de La Paz, Gulf of California. CICIMAR Oceánides, 272, 37-49.

Guindon S, Gascuel O 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52, 696-704.

Haines PE, Tomlinson RB, Thom BG. 2006. Morphometric assessment of intermittently open/closed coastal lagoons in New South Wales, Australia. Estuarine and Coastal Shelf Sciences 67, 321-332.

Hallegraeff GM 2002. Aquaculturist's guide to harmful Australian Microalgae. Hobart, School of Plant Science, University of Tasmania. 136 p.

Hinga, KR 1992. Co-occurrence of dinoflagellate blooms and high pH in marine enclosures. Marine Ecology Progress Series 86, 181-187.

Hoppenrath M 2000. Taxonomische und ökologische Untersuchungen von Flagellaten mariner Sande. PhD Thesis, Biologische Fakultät, Universität Hamburg, Germany 311 p.

Houdai T, Matsuoka S, Murata M, Satake M, Ota S, Oshima Y, Rhodes L 2001. Acetate labeling patterns of dinoflagellate polyketides, amphidinol 2, 3 and 4. Tetrahedron 57, 5551–5555.

Houdai T, Matsuoka S, Morsy N, Matsumori N, Satake M, Murata M 2005. Hairpin conformation of amphidinols possibly accounting for potent membrane permeabilizing activities. Tetrahedron. 61, 2795–2802.

Ismael AAH, Halim Y, Khalil AG 1999. Optimum growth conditions for *Amphidinium carterae* Hulburt from eutrophic waters in Alexandria Egypt. and its toxicity to the brine shrimp *Artemia salina*. Grana 38, 2-3, 179-185

Jones JB, Rhodes LL 1994. Suffocation of pilchards *Sardinops sagax*. by a green microalgal bloom in Wellington Harbour, New Zealand. New Zealand Journal of Marine and Freshwater Research, 28,379-383

Kamykowski D, Zentara SJ 1977. The diurnal vertical migration of motile phytoplankton through temperature gradients. Limnology and Oceanography 22, 148-151.

Katoh K, Misawa K, Kuma Ki, Miyata T 2002. MAFFT, a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30, 3059-3066.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. 2012. Geneious Basic, An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647-1649.

Kempton JW, Lewitus AJ, Deeds JR, Law JM, Place AR 2002. Toxicity of *Karlodinium veneficum* Dinophyceae. associated with a fish kill in a South Carolina brackish retention pond. Harmful Algae 1, 233-241.

Kitaya YL, Xiao A, Masuda T, Ozawa M, Tsuda, Omasa K 2008.. Effects of temperature, photosynthetic photon flux density, photoperiod and O2 and CO2 concentrations on growth rates of the symbiotic dinoflagellate, *Amphidinium* sp. Journal of Applied Phycology 20,737-742

Kubota T, Iinuma Y, Kobayashi J 2006. Cloning of polyketide synthase genes from amphidinolide-producing, dinoflagellate *Amphidinium* sp. Biol Pharm Bull 29, 1314-1318.

Kubota T, Tsuda M, Doi Y, Takahashi A, Nakamichi H, Ishibashi M, Fukushi E, Kawabata J, Kobayashi J 1998. Luteophanols B and C, new polyhydroxyl compounds from marine dinoflagellate *Amphidinium* sp. Tetrahedron 54, 14455-14464.

Kubota T, Takahashi A, Tsuda M, Kobayashi J. 2005. Luteophanol D, new polyhydroxyl metabolite from marine dinoflagellate *Amphidinium* sp. Marine Drugs 3, 113-118.

Lee JJ, Shpigel M, Freeman S, Zmora O, McLeod S, et al. 2003. Physiological ecology and possible control strategy of a toxic marine dinoflagellate, *Amphidinium* sp., from the benthos of a mariculture pond. Aquaculture 217, 351-371.

Monteith JL 1969. Light interception and radiative exchange in crop stands. In, Eastin JD, Haskins FA, Sullivan CY, Van Bavel CHM, editors. Physiological aspects of crop yield. Madison, American Society of Agronomy and Crop Science Society of America. pp. 89-116.

Morsy N, Matsuoka S, Houdai T, Matsumori N, Adachi S, Murata M, Iwashita T, Fujita T. 2005. Isolation and structure elucidation of a new amphidinol with a truncated polyhydroxyl chain from *Amphidinium klebsii*. Tetrahedron 61, 8606–8610.

Morsy N, Houdai T, Matsuoka S, Matsumori N, Adachi S, Oishi T, Murata M, Iwashita T, Fujita T. 2006. Structures of new amphidinols with truncated polyhydroxyl chain and their membrane-permeabilizing activities. Bioorganic and Medicinal Chemistry 14, 6548–6554.

Murray SA, Patterson DJ 2002. The benthic dinoflagellate genus *Amphidinium* in south-eastern Australian waters, including three new species. European Journal of Phycology 37, 279-298.

Murray SA, Garby T, Hoppenrath M, Neilan BA 2012. Genetic Diversity, Morphological Uniformity and Polyketide Production in Dinoflagellates *Amphidinium*, Dinoflagellata. PLoS One 76., e38253.

Murray SA, Flø Jørgensen M, Daugbjerg N, Rhodes L 2004. *Amphidinium* revisited. II. Resolving species boundaries in the *Amphidinium operculatum* species complex Dinophyceae., including the descriptions of *Amphidinium trulla* sp. nov. and *Amphidinium gibbosum* comb. nov. Journal of Phycology 40, 366-382.

Murray SA 2012. Microalgal Community Composition Assessment in Warringah Lagoons Final Report For Warringah Council. 17 p.

Nayak, BB, Karunasagar, I, Karunasagar I 1997. Influence of bacteria on growth and hemolysis production by the marine dinoflagellate *Amphidinium carterae*. Marine Biology 130, 35-39.

NSW Department of Primary Industry. 2010. Fish kills in NSW, http,//www.dpi.nsw.gov.au/data/assets/pdf_file/0004/402790/Fish-Kills-FAQ-August-2011.pdf. Accessed 3/7/13

Pagliara P, Caroppo C. 2012. Toxicity assessment of *Amphidinium carterae, Coolia cf monotis* and *Ostreopsis cf ovata* Dinophyta. isolated from the northern Ionian Sea Mediterranean Sea. Toxicon 60, 1203-1214.

Parslow, J. S., Hunter, J., Davidson, A., 1999. Estuarine eutrophication models. LWRRDC Occasional Paper 19/99 Urban Sub-Program.. Hobart, Tasmania, CSIRO Marine Research.112pp.

Paul GK, Matsumori N, Murata M, Tachibana K 1995. Isolation and chemical-structure of amphidinol-2, a potent hemolytic compound from marine dinoflagellate Amphidinium klebsii. Tetrahedron Letters 36, 6279-6282.

Paul GK, Matsumori N, Konoki K, Murata M, Tachibana K 1997. Chemical structures of amphidinols 5 and 6 isolated from marine dinoflagellate *Amphidinium klebsii* and their cholesterol-dependent membrane disruption. Journal of Marine Biotechnology 5, 124-128.

Peng J., Place A.R., Yoshida W., Anklin C., Hamann M.T., 2010. Structure and absolute configuration of karlotoxin-2, an ichthyotoxin from the marine dinoflagellate *Karlodinium veneficum*. Journal of the American Chemical Society 132, 3277-3279.

Place AR, Bowers HA, Bachvaroff TR, Adolf JE, Deeds JR, Sheng J 2012. *Karlodinium veneficum* – The little dinoflagellate with a big bite. Harmful Algae 14, 179-195.

Posada D, Crandall KA 1998. MODELTEST, testing the model of DNA substitution. Bioinformatics 14, 817-818.

Rhodes L, Smith KF, Munday R, Selwood AI, McNabb P, Holland P, Bottein M-Y 2010. Toxic dinoflagellates Dinophyceae. from Rarotonga, Cook Islands. Toxicon 56, 751-758.

Roach AC 1997. The effect of acid water inflow on estuarine benthic and fish communities in the Richmond River, N.S.W., Australia. Australian Journal of Ecotoxicology 3, 25–56.

Sampayo MA 1985. Encystment and excystment of a Portugese isolate of *Amphidinium carterae* in cultures. In, Anderson DM, White AW, Baden DG, editors. Toxic Dinoflagellates. Amsterdam, Elsevier. pp. 125-130.

Sammut J, Melville MD, Callinen RB, Frazer GC 1995. Estuarine acidification, impacts on aquatic biota of draining acid sulphate soils. Australian Geographic Studies 33, 89-100.21.

Satake M, Murata M, Yasumoto T, Fujita T, Naoki H 1991. *Amphidinol*, a polyhydroxypolyene antifungal agent with an unprecedented structure, from a marine dinoflagellate, *Amphininium klebsii*. Journal of the American Chemical Society 113, 9859-9861.

Schirmer K, Chan AGJ, Greenberg BM, Dixon DG, Bols NC 1997. Methodology for demonstrating and measuring the photocytotoxicity of fluoranthene to fish cells in culture. Toxicol In Vitro 11, 107-113.

Smayda TJ 1997. Harmful algal blooms, their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42, No. 5, Part 2, The Ecology and Oceanography of Harmful Algal Blooms, 1137-1153 pp.

ten Lohuis MR, Miller DJ 1998. Genetic transformation of dinoflagellates *Amphidinium* and *Symbiodinium*., expression of GUS in microalgae using heterologous promoter constructs. The Plant Journal 13, 427-435.

Tsuda M, Oguchi K, Iwamoto R, Okamoto Y, Fukushi E, Kawabata J, Ozawa T, Masuda A 2007. Iriomoteolides-1b and -1c, 20-membered macrolides from a marine dinoflagellate *Amphidinium* species. Journal of Natural Products 70, 1661-1663.

Van Wagoner RM, Deeds JR, Satake M, Ribeiro AA, Place AR, Wright JLC 2008. Isolation and characterization of karlotoxin 1, a new amphipathic toxin from *Karlodinium veneficum*. Tetrahedron Letters 49, 6457–6461.

Van Wagoner RM, Deeds JR, Tatters AO, Place AR, Tomas CR, Wright JLC 2010. Structure and relative potency of several karlotoxins from *Karlodinium veneficum*. Journal of Natural Products 73, 1360-1365.

Zimmermann LA 2006. Environmental regulation of toxin production, comparison of hemolytic activity of *Amphidinium carterae* and *Amphidinium klebsii*. MSc thesis. Wilmington, University of North Carolina. 83 p.55.

Figure Legends

Figure 1. (A) Map of the coast of New South Wales, Australia showing the location of Curl Curl Lagoon. (B) Map of Curl Curl Lagoon, showing the location of three sampling sites (1, 2, 3).

Figure 2. (A) Abundance of different species of phytoplankton observed at three sampling sites in Curl Curl Lagoon on 17th September 2012 during the *Amphidinium* bloom. (B) Mean and standard deviations of nutrient for samples and abundance of *Amphidinium carterae* taken on 17th September 2012 from three sampling sites in Curl Curl Lagoon.

Figure 3. Phylogenetic analysis using maximum likelihood of ITS gene sequences obtained from *Amphidinium* bloom sample. Support values are bootstrap values based on 500 replicates. Reference sequence names are followed by the strain number, if available and then accession number. Sequence denoted in bold letters was obtained during this study.

Figure 4. HPLC chromatography (Lower Panel) on a C8 reverse phase column of the methanol extracted material from the lyophilized bloom sample and the purified material (middle panel). The UV spectra for the purified material (Upper panel) is identical to that described for Luteophanol A [55].

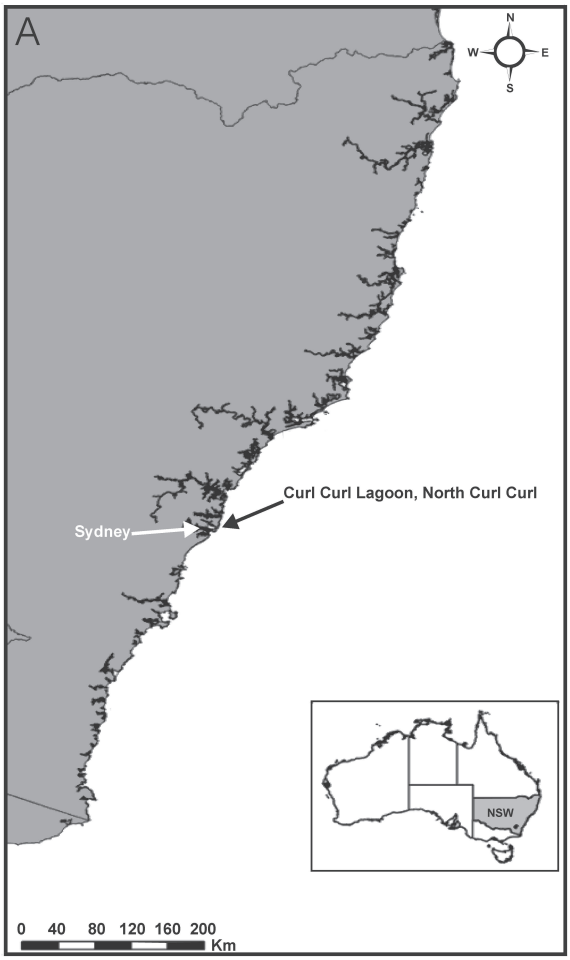
Figure 5. The mass spectra for the purified material is consistent with two congeners (base plus a hydroxylated form) that is sulphated similar to Luteophanol A.

Figure 6. Effect of a pure extract from the *Amphidinium carterae* bloom on viability of fish gill cells RTgill-W1. Gill cells showed a dramatic decrease of viability after exposure to 100 µg mL^{-1} for 2 hrs (48±2.7%), or 50 µg mL^{-1} for 6 hrs (36±8%). LC_{50} obtained for the extract purified from *A. carterae* from Curl Curl Lagoon was 95 and 23.5 µg mL^{-1}, at 2 and 6 hrs, respectively. Values represent the mean and error bars the standard deviation of cell viability from quadruplicate wells.

Supporting Information Legends

Figure S1. Dissolved oxygen (mg L^{-1}) at 9 sites in Curl Curl Lagoon, 1994-2000. Unpublished data collected by Warringah Council, Natural Environment Unit.

Figure


A
N
W
E
S
Curl Curl Lagoon, North Curl Curl
Sydney
NSW
0 40 80 120 160 200
Km

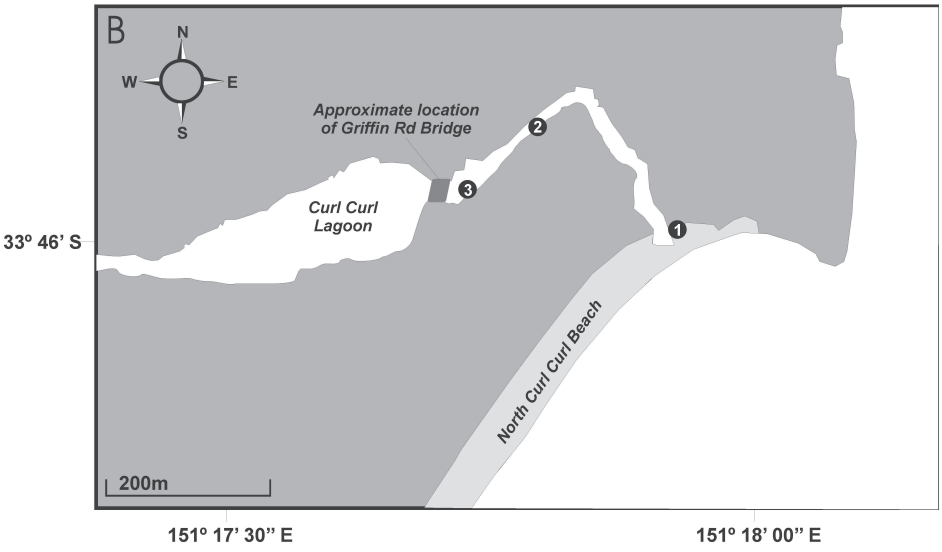
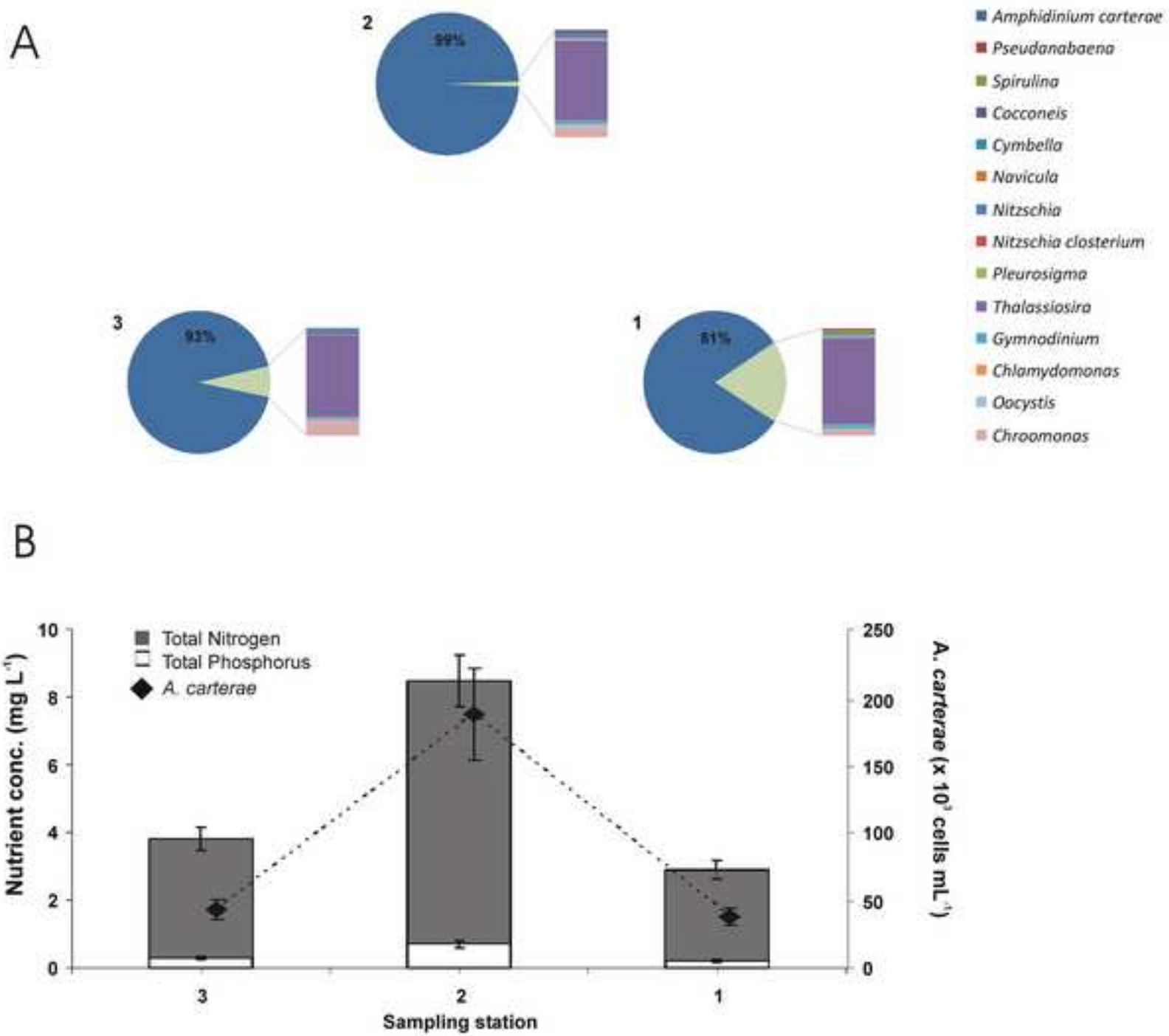
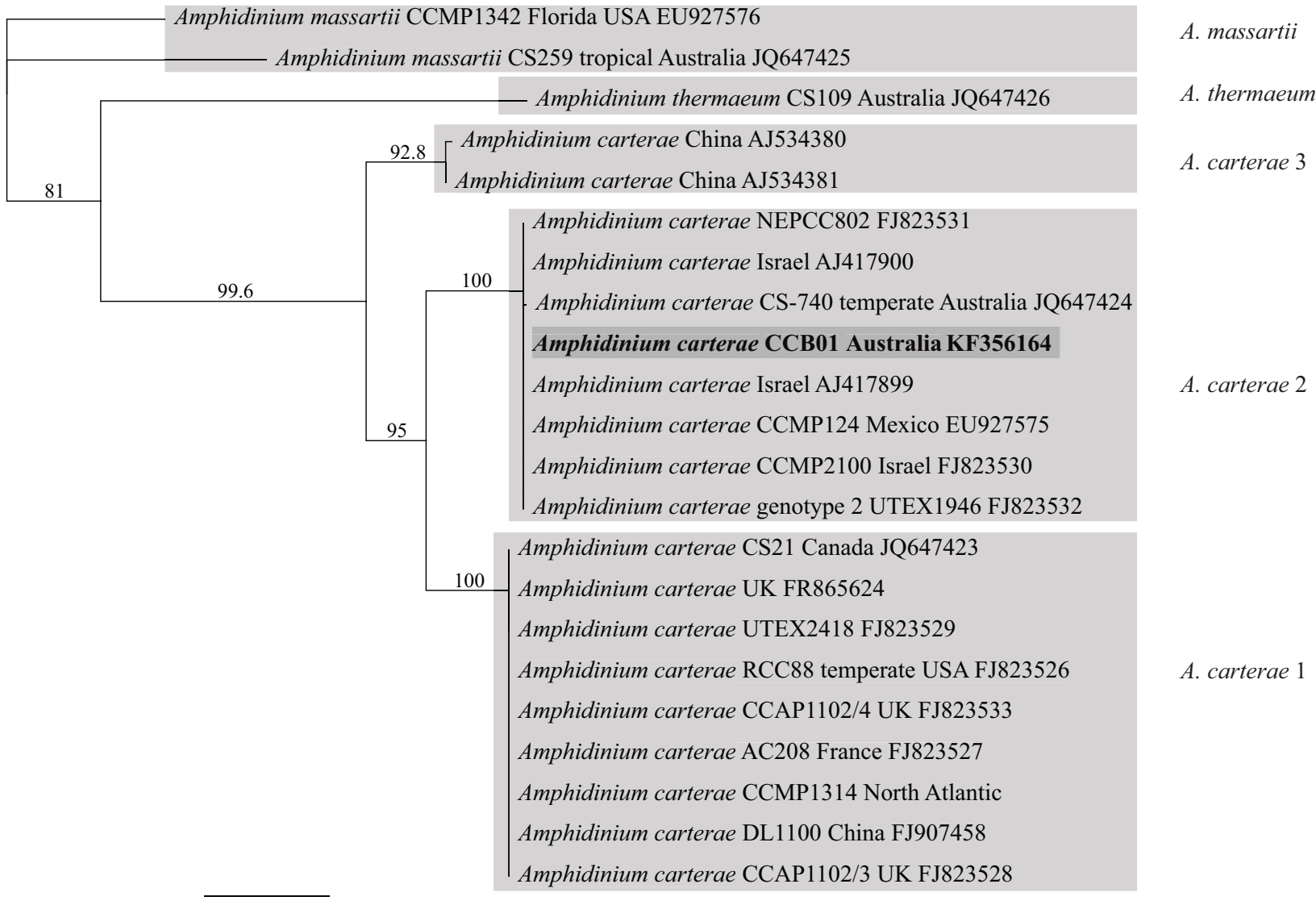

B
N
W
E
S
Approximate location
of Griffin Rd Bridge
Curl Curl
Lagoon
North Curl Curl Beach
200m
33° 46' S
151° 17' 30" E
151° 18' 00" E

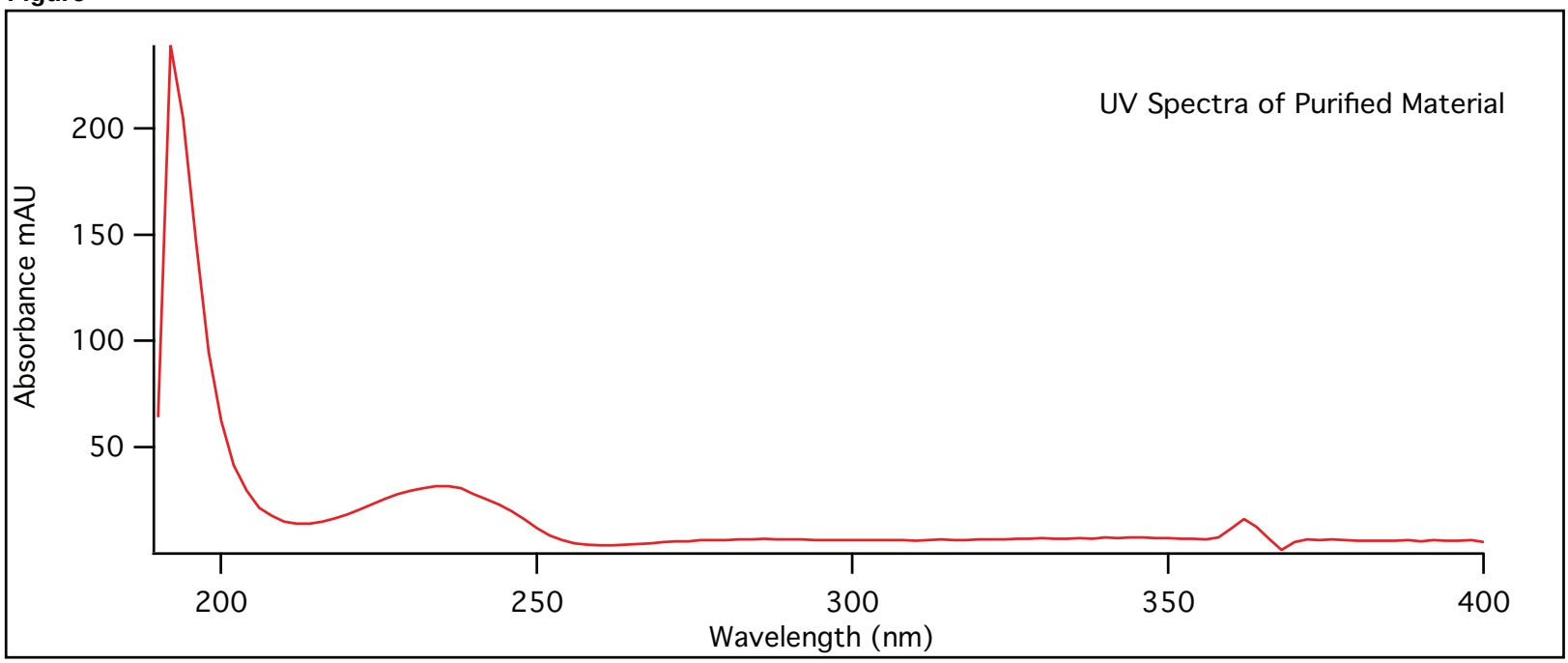
Figure
Click here to download high resolution image

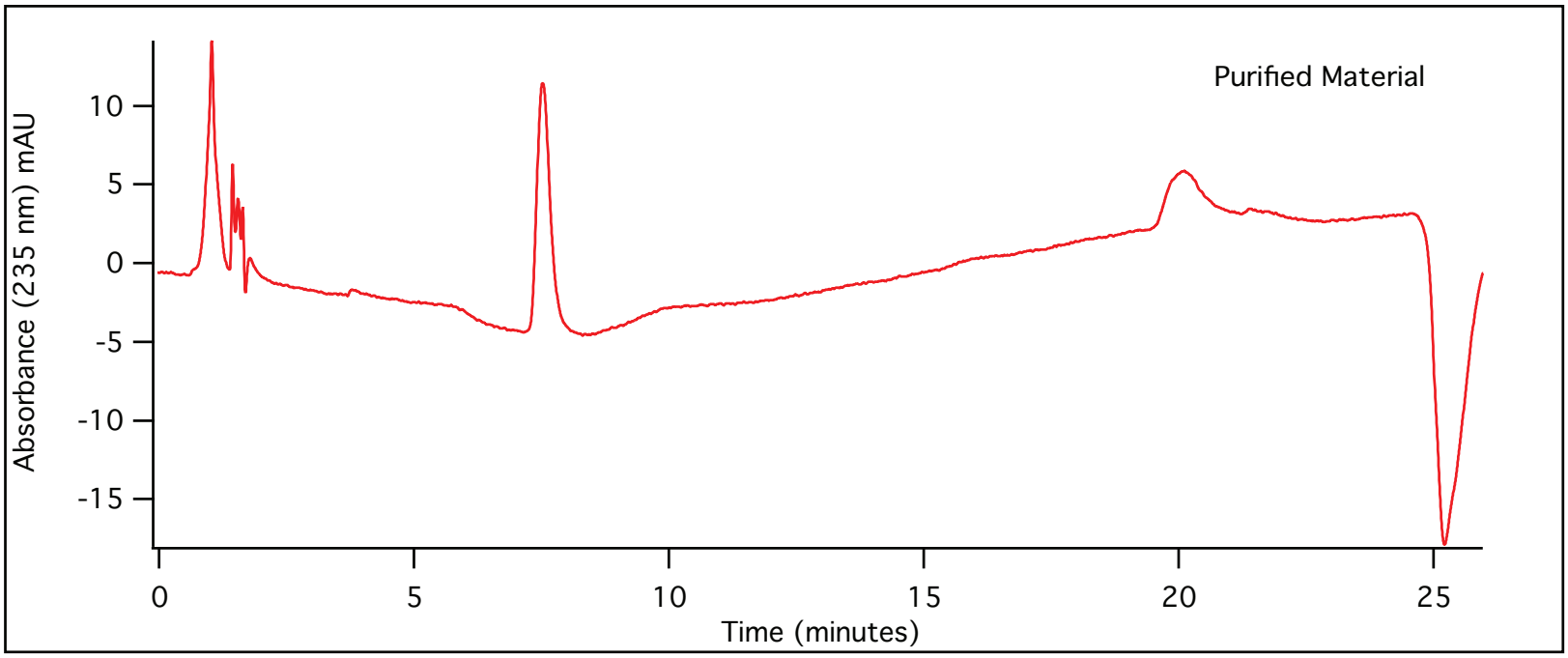


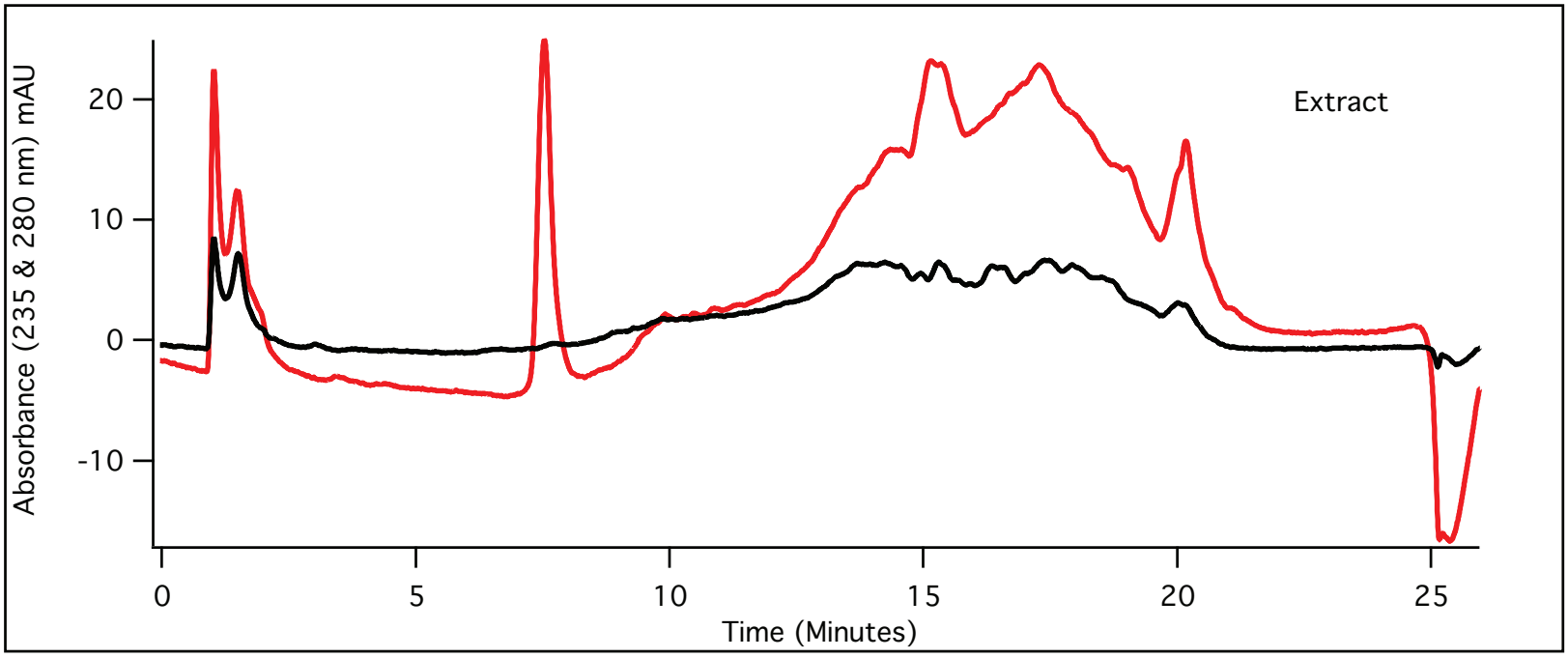
Figure


Amphidinium massartii CCMP1342 Florida USA EU927576
Amphidinium massartii CS259 tropical Australia JQ647425
A. massartii
Amphidinium thermaeum CS109 Australia JQ647426
A. thermaeum
92.8
Amphidinium carterae China AJ534380
Amphidinium carterae China AJ534381
A. carterae 3
81
Amphidinium carterae NEPCC802 FJ823531
Amphidinium carterae Israel AJ417900
100
99.6
Amphidinium carterae CS-740 temperate Australia JQ647424
***Amphidinium carterae* CCB01 Australia KF356164**
Amphidinium carterae Israel AJ417899
A. carterae 2
Amphidinium carterae CCMP124 Mexico EU927575
95
Amphidinium carterae CCMP2100 Israel FJ823530
Amphidinium carterae genotype 2 UTEX1946 FJ823532
Amphidinium carterae CS21 Canada JQ647423
100
Amphidinium carterae UK FR865624
Amphidinium carterae UTEX2418 FJ823529
Amphidinium carterae RCC88 temperate USA FJ823526
A. carterae 1
Amphidinium carterae CCAP1102/4 UK FJ823533
Amphidinium carterae AC208 France FJ823527
Amphidinium carterae CCMP1314 North Atlantic
Amphidinium carterae DL1100 China FJ907458
Amphidinium carterae CCAP1102/3 UK FJ823528
0.2

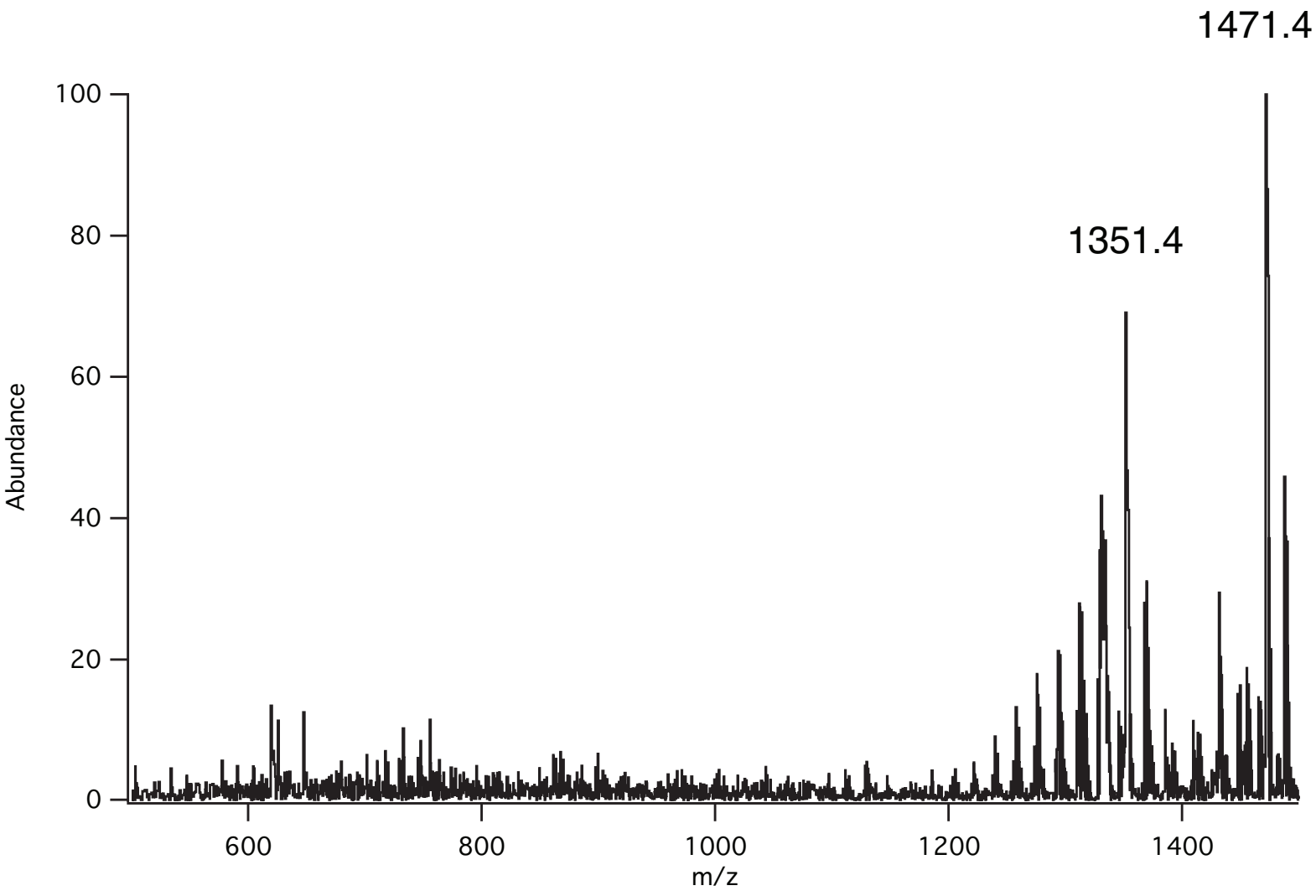
Figure


UV Spectra of Purified Material
Absorbance mAU
200
150
100
50
200
250
300
350
400
Wavelength (nm)


Purified Material
Absorbance (235 nm) mAU
10
5
0
-5
-10
-15
0
5
10
15
20
25
Time (minutes)

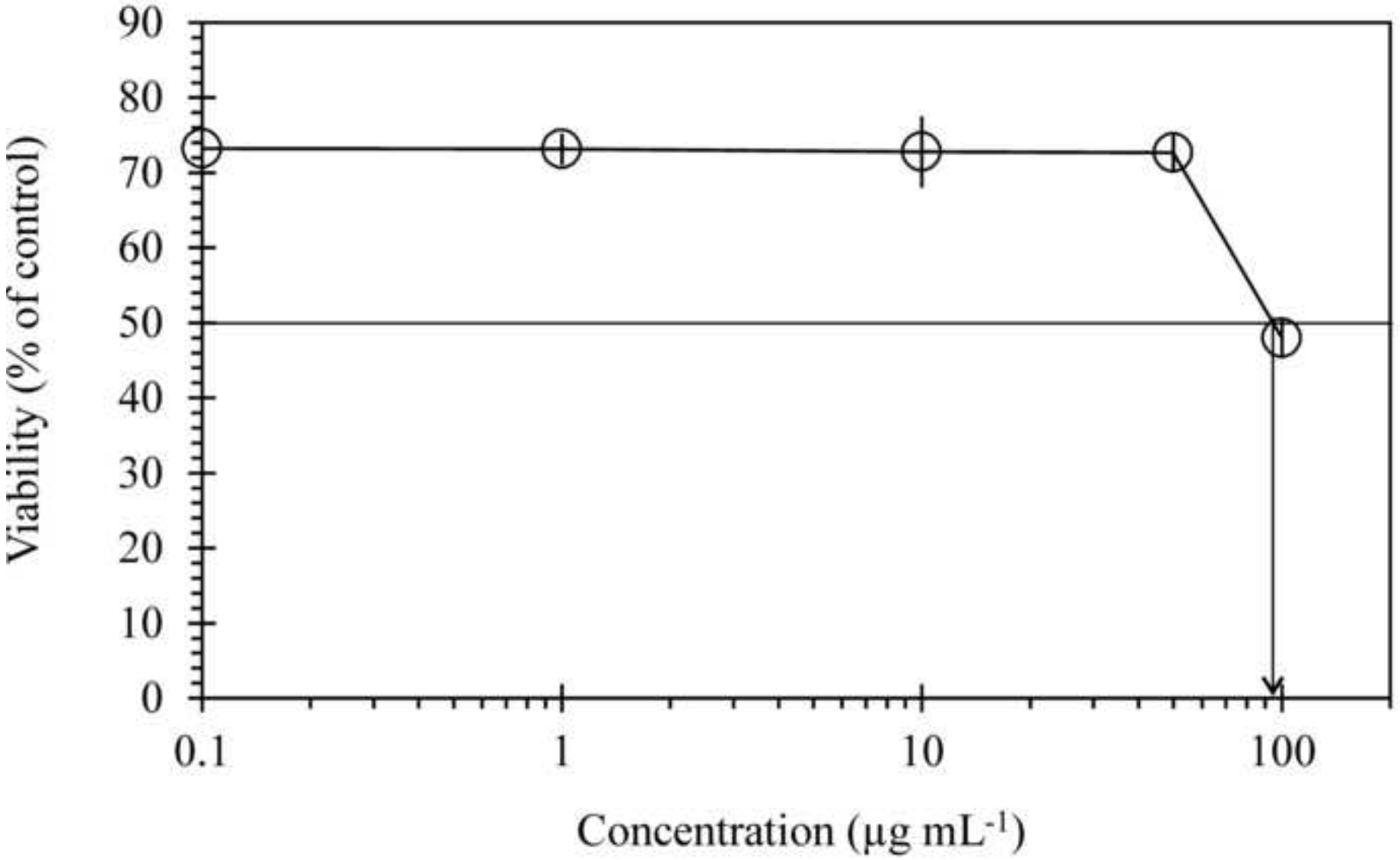

Extract
Absorbance (235 & 280 nm) mAU
20
10
0
-10
0
5
10
15
20
25
Time (Minutes)

Figure


1471.4
1351.4
100
80
60
40
20
0
Abundance
600
800
1000
1200
1400
m/z

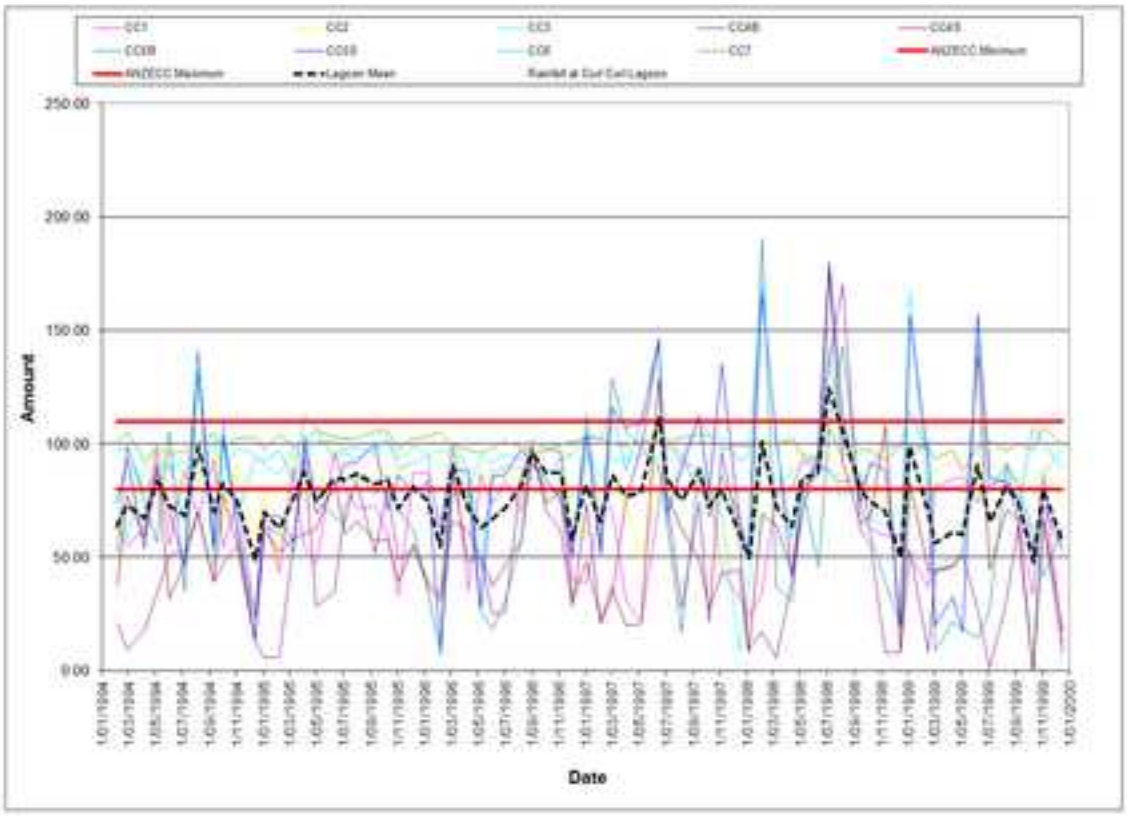
Figure

Click here to download high resolution image



Supplementary Material

Click here to download high resolution image



Supplementary Figure 1. Dissolved oxygen (mg /L) at 9 sites in Curl Curl Lagoon, 1994-2000. Unpublished data collected by Warringah Council, Natural Environment Unit.

Supplementary Table 1. Summary of dissolved oxygen data from Curl Curl Lagoon, 1994-2000.

Site	CC1	CC2	CC3	CC4B	CC4S	CC5B	CC5S	CC6	CC7	Lagoon Mean
Count	71	71	71	71	71	71	71	71	71	71
Mean	68.13	82.11	86.05	45.93	58.07	67.47	81.81	93.35	99.86	76.31
Minimum	12.30	44.10	0.00	0.00	0.00	6.80	10.50	44.20	88.10	47.83
Maximum	161.20	103.50	167.20	170.30	176.20	190.70	180.00	169.60	107.60	124.52
Standard Deviation	22.90	13.73	21.42	34.44	29.70	34.48	34.81	22.63	3.68	14.71
Variance	524.61	188.52	458.73	1186.11	882.29	1188.35	1211.96	512.18	13.57	216.42