Occurrence of okadaic acid in the feeding grounds of dugongs (Dugong dugon) and green turtles (Chelonia mydas) in Moreton Bay, Australia

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Abstract

Okadaic acid (OA) is a diarrhetic shellfish poison (DSP) produced by a number of marine organisms including the benthic dinoflagellate Prorocentrum lima, which are often found on seagrass. As seagrass forms the basis of the diet of dugong (Dugong dugon) and green turtle (Chelonia mydas), these herbivores may potentially be exposed to OA through ingestion of P. lima found on the seagrass. In this study, the abundance of epiphytic P. lima, on seagrass, and the concentration of OA produced by these epiphytic dinoflagellates was measured in Moreton Bay, Queensland, Australia. Prorocentrum lima and OA were found on all four species of seagrass collected. OA was detected in epiphytic material collected from seagrass, with a maximum of 460 ng OA/$kg_{(wwtSG)}$ found on Halophila spinulosa . From this information, the estimated maximum daily intake of OA by an adult dugong consuming 40 $kg_{(wwtSG)}$ /day was 18,400 ng/day, and an adult turtle consuming 2 $kg_{(wwtSG)}$ /day was 920 ng/day. Analysis by HPLC/MS/MS of 54 stranded dugongs and 19 stranded turtles did not yield OA above the detection limit of 10,000 ng/$kg_{(animal\ tissue)}$. OA was found on seagrass, however it was not detected in the tissue samples of dugongs and turtles.

Keywords: Prorocentrum lima, seagrass, Moreton Bay, okadaic acid, dugong, green turtle

1. Introduction

Okadaic acid (OA) is a lipophilic marine algal toxin, which was first extracted from sponges Halichondria okadai and H. melanodocia (Tachibana, 1981) and was later found to be identical to the toxin isolated from the dinoflagellates genus

Prorocentrum and Dinophysis (Murakami et al., 1982; Yasumoto et al., 1985; Yasumoto and Murata, 1990; Carmody, 1996). OA is one of the main toxins responsible for diarrhetic shellfish poisoning (DSP) (Murata, 1982), a term used to describe the rapid onset of gastrointestinal symptoms such as vomiting and diarrhoea in people who have consumed toxic shellfish (Amzil et al., 1992; James, 1999; Souto et al., 2001; Van Dolah et al., 2003). Prorocentrum spp. are single cell benthic epiphytes (Takai et al., 1987; Bialojan and Takai, 1988; Ishihara, 1989; Arias et al., 1993) that are often found on seagrass and macroalgae (Koike et al., 1998; Bouaicha, 2001). Amongst the OA-producing Prorocentrum species, P. lima is one of the main species known to produce OA (Morton and Tindall, 1995; Van Dolah, 2000) and, being a photosynthetic benthic organism, P. lima occurs on substrates such as seagrass and macroalgae where it has access to light (Morton et al., 1998).

Although OA has not been known to cause death when orally consumed, the LD_{50} for OA in mice injected intravenously (i.v.) has been reported to be 192 μg/kg (Matias and Creppy, 1996). Chronic exposure to OA promotes tumor development on the skin of laboratory animals by initiating hyper-phosphorylation (Fujiki, 1992). This is achieved by potently inhibiting three of the four protein serine/threonine phosphatases type 1, 2A and 2B (PP1, PP2A, PP2B) (Takai et al., 1987; Bialojan and Takai, 1988; Ishihara, 1989; Arias et al., 1993). Suganuma (1992) demonstrated that when 10 μg/day of OA is administered in rat drinking water for nine weeks, it can cause neoplastic changes in the glandular stomach. Another way in which OA could potentially lead to tumour promotion is by DNA hypermethylation which may lead to silencing of tumour suppressor genes (Creppy et al., 2002). Additionally, OA has also been shown to induce ornithine decarboxylase (ODC) in mice, which is a significant step in tumour promotion (Suganuma et al., 1992).

As seagrass is the primary food source of dugongs (Dugong dugon) and green turtles (Chelonia mydas), it is hypothesised that these vertebrate herbivores may be exposed to OA produced by P. lima found epiphytically on seagrass. In Moreton Bay, Australia, both green turtles and dugong are protected under the Australian Environmental Protection and Biodiversity Act 1999 (Bryden et al., 1998; Read and Limpus, 2002). Halophila ovalis is the favoured species of seagrass by these herbivores, however, they also feed on Halodule uninervis, Zostera muelleri and Halophila spinulosa (Lanyon, 1991; Read and Limpus, 2002). The potential exposure of green turtles to OA was assessed in a previous study through determination of P. lima and other Prorocentrum species abundance on common macroalgal food sources in Hawaii (Landsberg et al., 1999). The exposure to OA was investigated using the protein phosphate inhibition assay and demonstrated that presumptive OA concentration in kidneys of turtles ranged from 26 to 670 µg/g (26,000 to 670,000 µg/kg). The Hawaiian study examined the potential exposure of green turtles to OA in a region where turtles feed predominantly on a macroalgal diet.

To date, no studies have assessed the potential exposure of dugongs to OA. Since dugongs feed on seagrass, it is likely that dugongs are ingesting epiphytic microalgae that are known to occur on the seagrass and may consequently be exposed to the toxins they produce. Prorocentrum lima, P. micans and P. minimum have previously been observed in Moreton Bay (Heil, 1998); however, their toxicity has not been tested. This study aims to assess the presence of P. lima on seagrass collected in Moreton Bay and to quantify the production of OA by these microalgae. The potential exposure to OA is then estimated in turtles and dugong based on known feeding rates and the actual exposure assessed by measuring the OA concentration in tissues from dead animals.

2. Materials and Methods

2.1 Seagrass collection

2.1.1. Field sites and seagrass collection

Four species of seagrass were collected from three separate sites within Moreton Bay (Fig. 1). The sites were selected based on the known high occurrence of dugong and green turtle foraging in these areas (Limpus et al., 1994; Lanyon, 2003). Site one (Myora: S 27°28'.21.09", E 153° 24'27.24") is approximately 1 km west of North Stradbroke Island and receives terrigenous freshwater run-off from North Stradbroke Is., but is also flushed daily on the tidal cycle with oceanic water entering from Rainbow Channel. Site two (Palmer Passage: S27° 26'49.14"835, E153° 23'49.74") is approximately 4 km west of North Stradbroke Is. and characterized by typically clear water due to daily flushing with the tides. Site three (Rous Channel: S27° 24'47.72", E153° 22'20.32") is located on a bank adjacent to the Rous Channel and is characterized by clear water due to daily flushing with oceanic water. Dugongs have been seen to aggregate in large numbers at this last site (pers. obs.).

Seagrass sampling was conducted on a monthly basis between February 2004 and June 2005. Water temperature was recorded at each collection. Where available, approximately 50 g seagrass wet weight (wwtSG) of each seagrass species were collected from depths of 1-1.5 m at low tide). To avoid dislodging the epiphytic microalgae, individual leaves were carefully collected by hand underwater and gently brought to the surface. Samples of each seagrass species were collected in separate resealable plastic bags with 50 – 200 mL of seawater. These were maintained on ice until further processing.

101

102 2.1.2. Sample processing

103 Seagrass samples were returned to the laboratory where they were vigorously
104 shaken twenty times to dislodge epiphytic material (Morton and Tindall, 1995;
105 Landsberg et al., 1999). The water was carefully decanted from the seagrass and the
106 volume of water and seagrass wet weight noted. Each water sample was gently shaken
107 to resuspend particulate material and 15 mL aliquots were preserved in Lugol's iodine
108 for microscopic observation. The remaining water was collected for OA extraction of
109 epiphyte material dislodged from seagrass and collected in the water samples.

110

111 2.1.3. Prorocentrum lima abundance

112 The number of P. lima per gram of seagrass (wwt) was determined by
113 examining three 1 mL aliquots of water with suspended epiphytic material from
114 shaken seagrass, preserved in Lugol's iodine. Each aliquot was examined using phase
115 contrast microscopy (Nikon Labphot; Graticules, UK) at 400x magnification. P. lima
116 were identified as per Faust (2002). The entire sample was scanned and counts made
117 of all P. lima observed on the slide. The abundance of P. lima cells per volume of sea
118 water was extrapolated to obtain the number of cells per wet weight of seagrass.
119 Prorocentrum species other than P. lima were not recorded during this study.

120

121 2.1.4. Okadaic acid extraction and analysis

122 Seawater in which seagrass had been shaken was centrifuged at 1500 g for ten
123 minutes, and the supernatant discarded. Pellets were boiled at 100° C for five minutes
124 to lyse microalgal cells, releasing toxins, and to stop enzymes from degrading the
125 toxins (Pan et al., 1999). The pellet was extracted in 2 mL 80 % methanol, evaporated

126 under N_2, re-dissolved in 200 μL 80 % methanol and filtered for High Performance
127 Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry (HPLC-MS/MS)
128 analysis (Lee et al., 1987; Quilliam and Wright, 1995; Quilliam, 1996).
129
130 **2.2 Dugongs and turtles**
131 2.2.1. Sample collection
132 Tissue samples from dead stranded dugongs and turtles found in Moreton Bay
133 were received by Queensland Environmental Protection Agency (QEPA), Queensland
134 Health Pathology and Scientific Services (QHPSS) and The University of Queensland
135 (UQ). Tissue samples were stored at -20°C prior to extraction in either plastic
136 containers or aluminium foil. A total of 48 tissue samples from 41 individual dugongs
137 were obtained (19 blubber samples, 19 muscle samples, two liver samples, six diet
138 samples and two stool samples). In addition, pectoral muscle from 19 green turtles
139 was collected. Tissue samples were collected at the time of necropsy by the veterinary
140 staff at the University of Queensland Veterinary School or the Department of Primary
141 Industries, Brisbane. No mass mortality events occurred during the sampling periods
142 and the cause of death of many of the animals was not established. All tissue samples
143 were collected between August 2000 and August 2004, with most stranding occurring
144 in the winter / spring months, and all from within Moreton Bay and Sunshine Coast
145 area, Southeast Queensland.
146
147 2.2.2. Toxin extraction and analysis
148 The extraction of OA from turtle and dugong tissues followed the method
149 previously developed by Lee (1987), with some alteration (Lee et al., 1987; Marr et
150 al., 1994; Lefebvre et al., 2002). Four grams of wet tissue were homogenised in 16

mL 80 % methanol. The homogenate was vortexed, sonicated and homogenised again to ensure complete breakdown of cells. It was then centrifuged for 20 minutes at 2700 g. The supernatant was filtered for HPLC-MS/MS analysis. To verify the extraction efficiency, two dugong muscle tissue samples were spiked with 185 µg/kg OA prior to the centrifugation step.

All seagrass epiphyte, dugong and turtle extracts were analysed using an AB/Sciex API 300 mass spectrometer (Applied Biosystems, Concord, Canada) coupled to a Perkin Elmer 200 series HPLC system (Perkin Elmer, Norwalk, USA) by a high flow Electrospray interface (Turbo-Ionspray) (Burgess, 2003). Analytes were separated on an Altima C18 column (150 x 4.6mm, Alltech Associates, Deerfield, IL.) using an acetonitrile 2mM ammonium formate 0.1% formic acid gradient at a flow rate of 0.8 mL/min. The mass spectrometer was operated in the negative ion mode for OA. Samples were compared against certified reference material (NRC-CNRC, Halifax).

2.3. Statistics

Statistical analysis was undertaken using SigmaStat 3.11 (Systat Software, California, USA). The concentration of OA per wet weight of seagrass was calculated for each seagrass species by dividing the OA concentration by the volume of water used for extraction and then multiplying by the wet weight of seagrass collected. For this reason, all OA concentrations will be provided on a wet weight basis. OA production per cell was determined by dividing the OA concentration by abundance of P. lima counted in each sample. Pearson product moment correlations were used to assess the relationships between OA concentration and the abundance of P. lima cells, water temperature and the concentration of OA in seagrass epiphytic material. OA

concentration data failed the test for homogeneity of variance and as such a Kruskal-Wallis One Way Analysis of Variance on Ranks was used to compare OA concentration on each species of seagrass and the site at which samples were collected. Where a significant result was obtained, a post-hoc pairwise multiple comparison procedure (Dunn's Method) was used to assess each pair.

Estimated daily intake of OA for dugongs and turtles was calculated by multiplying the OA concentration measured for each seagrass species by the daily consumption of seagrass by the animals based on a 400 kg dugong consuming ~40 $kg_{(wwtSG)}$ /day (Lanyon, 1991) and a 60 kg turtle consuming ~2 $kg_{(wwtSG)}$ /day (Bjorndal, 1980). The average daily intake per kg body weight of animal was calculated by dividing the estimated daily intake by the average body weight. This value was then used as the estimation of OA in tissue samples.

3. Results

3.1 Prorocentrum lima abundance and algal toxins from seagrass samples

Prorocentrum lima was found on all four species of seagrass and at all three sites examined in Moreton Bay between February 2004 and June 2005. Similarly, OA was detected in seagrass epiphyte extracts collected at all sites and from all seagrass species. There was a significant correlation between the P. lima cell abundance and the OA concentration (Pearson correlation: $r = 0.40$; $p < 0.001$; Fig 2), with the average OA production per cell being 0.24 (± 0.04) pg/cell.

Two of the four seagrass species examined were found at all sites. These were Z. muelleri and H. ovalis. In addition to these two species, C. serrulata was found at Myora and Pumicestone Passage, while H. spinulosa was only found at Rous Channel.

H. spinulosa samples had significantly more OA present (than the other seagrass species (Kruskal-Wallis One Way Analysis of Variance on Ranks: H = 13.875, df = 3, P = 0.003: Fig. 3.1); however, this species was only observed at Rous Channel where a significantly higher concentration of OA was observed in seagrass epiphytes (Kruskal-Wallis One Way Analysis of Variance on Ranks: H = 19.505, df = 2, P <0.001: Fig. 3.2). To elucidate whether these differences were driven by site or species, we examined the two species that were found at all sites (Z. muelleri and H. ovalis) and found that while there was not a significant difference between the concentration of OA found on these two species (Kruskal-Wallis One Way Analysis of Variance on Ranks: H = 0.336, df = 1, P = 0.562; Fig. 3.1), there was a significant effect of site when only these two species were considered (Kruskal-Wallis One Way Analysis of Variance on Ranks: H = 8.826, df = 2, P = 0.012; Fig. 3.3).

During the sampling periods, temperature of the water varied between 16 and 27 °C. OA concentration was significantly correlated with the rise in temperature (Pearson correlation: r = 0.24, p = 0.016); however, the number of P. lima cells present in seagrass epiphyte material was not significantly correlated to water temperature (Pearson correlation: r = 0.02, p = 0.813). The peak cell abundance and OA concentration were seen in November 2004, during spring (Fig. 4), at which time the water temperature was at 24°C. Both cell abundance and OA concentration also showed a slight increase during winter months (water temperature 16-17 °C).

3.2 Exposure of dugongs and turtles to OA

H. spinulosa was found to be associated with the highest concentration of OA; therefore, the maximum potential exposure of dugongs and turtles to OA were calculated using the maximum concentration of OA extracted from H. spinulosa. For

estimating the mean OA exposure, the mean concentration of OA extracted from H. ovalis was used since H. ovalis is known to be the preferred species of seagrass for both dugongs and turtles in this area (Lanyon, 1991; Read and Limpus, 2002).

The maximum and mean daily intakes (DI) of OA were calculated for dugongs and turtles (Table 1). The estimated maximum DI by dugongs was 2,440 ng/day and the mean 18,400 ng/day. From the estimated DIs, the maximum daily intake per body weight of a 400kg dugong per kilogram of tissue was estimated to be 46 ng/day/$kg_{(dugong)}$ and mean to be 6 ng/day/$kg_{(dugong)}$. Similarly, a 60 kg green turtle is known to consume, on average, 218 g/seagrass/$day_{(dwtSG)}$ (Bjorndal, 1980), which equates to approximately 2 kg/seagrass/$day_{(wwtSG)}$ using a wet:dry conversion of 8.75 (Gaus, 2002). From this, it can then be estimated that these green turtles are consuming a maximum of 920 ng/day and a mean of 120 ng/day. The maximum and mean OA concentrations per body weight are 15 ng/day/$kg_{(turtle)}$ and 2 ng/day/$kg_{(turtle)}$ respectively.

3.3. OA in tissue samples of dugongs and turtles

OA was not detected from any of the tissue samples from dugongs or turtles using HPLC/MS/MS analysis which has a detection limit of 10,000 ng/$kg_{(animal\ tissue)}$. Concurrent spike and recovery quality assurance samples demonstrated 96.0 (± 3.2) % recovery rate from dugong muscle tissue.

4. Discussion

Prorocentrum lima and OA were detected on epiphyte material collected from seagrass in Moreton Bay. The significant correlation between P. lima cell abundance

and OA concentration suggests that the P. lima observed on the seagrass was the dominant producer of OA.

The concentration of OA in seagrass epiphytic material varied significantly between the seagrass species from which they were collected, with H. spinulosa found to have significantly more OA present in epiphytic material per wet weight of seagrass (Fig. 3.1). In addition, this species of seagrass was only found at Rous Channel, the site found to have significantly higher concentrations of OA when only Z. capricorni and H. ovalis (the two species found at all sites) were considered (Fig. 3.3). This suggests that the environmental conditions at the Rous Channel site may promote P. lima growth and / or OA production.

The difference in OA production around North Stradbroke Island is similar to studies conducted around Heron Island, in which presence and toxicity of P. lima have been previously documented (Morton and Tindall, 1995; Morton, 1998). Morton and Tindall showed that toxin production by P. lima varied significantly at different sites around the island, implying there may be different strains of P. lima within the same area, which could lead to varying levels of OA production.

Environmental factors such as light, nutrient availability, salinity and temperature may effect the growth of P. lima and OA production (McLachlan et al., 1994; Morton et al., 1994; Souto et al., 2001). In the present study, OA concentration in seagrass epiphytes on a seagrass weight basis, was significantly correlated with water temperature; however, cell abundance was not. This suggests that temperature may be the driver for OA production, while cell abundance may be more greatly affected by nutrient or light availability.

In the current study, P. lima was observed in seagrass epiphyte samples throughout the year with very little fluctuation in abundance; however, OA production peaked in the warmer summer and autumn months with the highest concentrations observed at 24 ºC. This is an indication of potential higher exposure of OA in summer for the dugongs and turtles.

Despite the presence of OA in seagrass epiphyte samples throughout the year, OA was not detected in dugong or turtle tissue samples from animals thought to have been feeding in the region where P. lima were observed. There may be several explanations for this. Firstly, it is possible that while P. lima was ingested, the OA was not absorbed and assimilated during digestion. In addition, we estimated that the likely average exposure to OA in a dugong or a turtle consuming H. ovalis from any of the sites we assessed was 2,440 ng/day and 120 ng/day respectively. Based on this estimated intake, and assuming 100% uptake through digestion with no metabolic breakdown or excretion and uniform distribution through tissue, the expected concentration in tissue after a twelve month average accumulated exposure would be 2,226 ng/kg$_{(dugong)}$ or 73 ng/kg$_{(turtle)}$. These estimates are well below the detection limit (10,000 ng/kg$_{(tissue)}$) available using HPLC/MS/MS. Also, as the animals that were available for analysis were all stranded animals and, hence, potentially sick prior to stranding, it is possible that the animals were not foraging normally prior to stranding. In addition, these animals were collected from various areas around the bay, and, as location appears to influence the amount of OA present in samples (Fig. 3), these animals may have been in residence in areas where OA exposure was less than estimated. Furthermore, since samples used in this assessment were collected for other purposes, limited tissue types were available for analysis. Had more turtle gastro-

296 intestine kidney or liver samples been available, results from these tissue types may
297 have yielded higher concentration of OA (Toyofuku, 2006).

298 A previous study in Hawaii estimated that the presence of OA in green turtle
299 kidney ranged between 24 - 670 $\mu g/g_{(tissue)}$ using a protein phosphate inhibition assay
300 (PPIA) (Landsberg et al., 1999). This estimate is much greater than the HPLC/MS/MS
301 detection limit determined in the current study even though the mean cell abundances
302 were similar (current study: 247.7 cells/$g_{(wwtSG)}$; Kaneohe Bay: 239.1
303 cells/$g_{(wwtSG/algae)}$). The major difference between these sites is the substrate on which
304 the turtles forage. In Moreton Bay turtles predominantly feed on seagrass with small
305 amounts of macroalgae (Read and Limpus, 2002), while turtles in Kaneohe Bay feed
306 predominantly on macroalgae supplemented with small amounts of seagrass (Arthur,
307 2006). OA in seagrass epiphyte material was not measured in the Hawaiian study and
308 since production of OA by P. lima is known to vary with environmental factors,
309 P. lima in Hawaii may have been producing a greater concentration of OA than those
310 in Moreton Bay. This difference in OA production per P. lima cells has also been
311 observed between this study (0.24 pg/cell) and that conducted in waters around Heron
312 Island by Morton and Tindall in 1995 (5.88 pg/cell).

313 Another major difference between the two studies is the method used to
314 estimate the amount of OA in turtle tissue. The PPIA is an indirect measure of OA, as
315 it is non-specific for the suite of protein phosphate inhibitors (Gehringer, 2004). Since
316 there are other natural toxins known to inhibit protein phosphatases, such as
317 microcystins and nodularin, produced by freshwater and marine cyanobacteria,
318 respectively (Honkanen et al., 1994), using PPIA alone could provide a false positive
319 result for OA. The common tropical marine cyanobacterium, Trichodesmium

320 erythraeum has been shown to produce microcystin-like compounds which inhibit
321 protein phosphatases (Shaw et al., 2001) and as such, an organism other than
322 Prorocentrum spp. may have been the source of the protein phosphatise inhibiting
323 compound in the Hawaiian study.

324 The potential exposure of dugong and turtle to OA that we have identified here
325 indicates that these large herbivores may be impacted by the deleterious effects of this
326 toxin. It has been suggested that naturally produced tumour promoting compounds
327 such as OA may play a role in green turtle fibropapilloma (FP) (Landsberg et al.,
328 1999; Arthur et al., 2006), a disease in which benign tumours grow both internally
329 and externally in marine turtles (Herbst, 1994). Although there are no records of
330 tumours in dugongs, these animals are likely to be consuming OA, and the presence
331 of OA producing P. lima in Moreton Bay provides a potential route of exposure to OA
332 for seagrass consumers. Hence long term risks for these animals are potentially an
333 issue. Carcinogenic chemicals, including persistent organic pollutants have been
334 determined in dugong tissue from Moreton Bay (Gaus, 2002), and the presence of
335 tumour promoters such as OA have the capacity to increase the risk of tumour
336 formation to an undetermined degree.

337 The metabolism of OA in marine mammals and reptiles is unknown, and
338 limited studies have been done on laboratory mammals. Matias et al. (1999) found
339 that when mice were orally dosed with 50 µg/kg OA, traces of the toxin were found in
340 all tissues. However, 37% were found in the intestine content (Matias et al., 1999).
341 Other studies also support the theory that the target organ for OA is the small intestine
342 (Ito, 2002). Although dugongs are also mammals, their metabolism would be slower
343 than rodents and they are also known to be a post-gastric fermenter (Lanyon, 1991).

Being a reptile, turtles may differ even further from laboratory rodents. For these reasons, it is difficult to extrapolate bioaccumulation and metabolism of OA in dugongs and turtles from laboratory studies on rodents.

This is the first time that OA has been detected in epiphytic material from seagrass collected in Moreton Bay. The presence of OA on seagrass suggests that large marine herbivores such as dugong and turtle may be exposed to these compounds throughout the year. However, no mass die-offs have been observed in the past (Haines and Limpus, 2000; Haines and Limpus, 2002), and although the presence of FP in the turtle population is high in some areas of the Bay (Limpus and Miller, 1994; Aguirre et al., 1999), there is at this stage, no direct evidence to suggest that OA poses a threat to these endangered species. However, the high detection limit using HPLC/MS/MS may mean that other techniques such as the non-specific PPIA in association with cell counts and OA analysis may be required to monitor OA exposure in the future.

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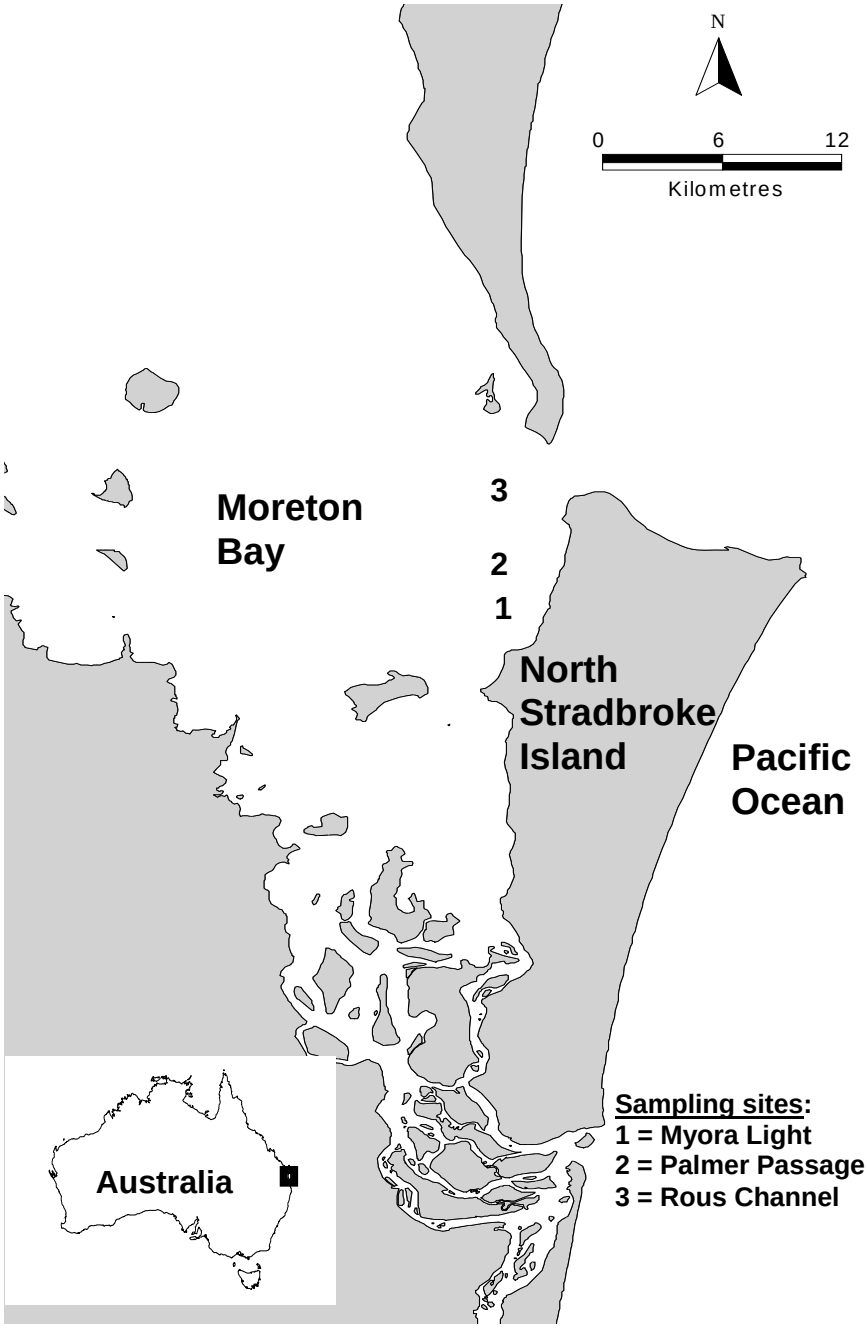
530

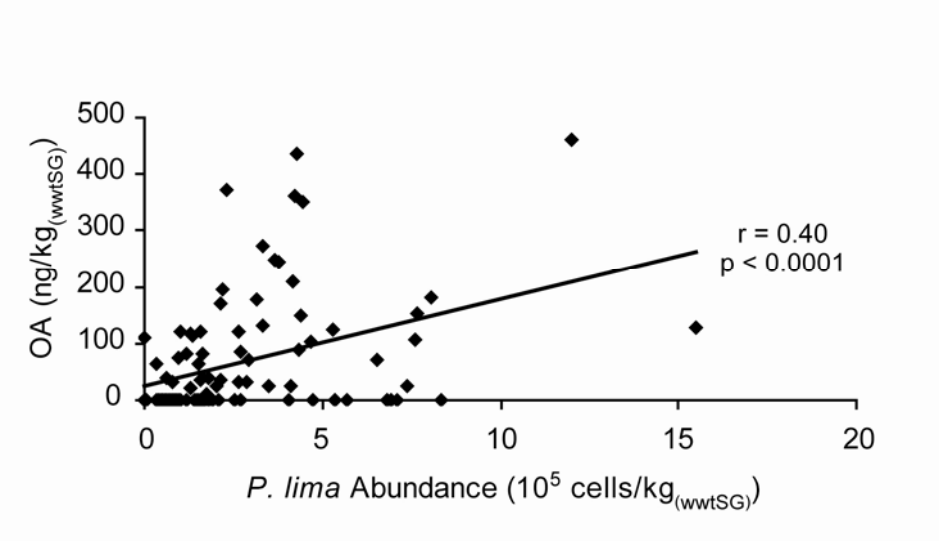
Table 1. Estimated maximum and mean okadaic acid (OA) daily exposure by dugongs and turtles was based on a maximum OA concentration observed on H. spinulosa (460 ng/$kg_{(wwtSG)}$), a mean concentration (60 ng/$kg_{(wwtSG)}$) detected in epiphyte material on H. ovalis and a daily seagrass intake of 40 $kg_{(wwtSG)}$ /day and 2$kg_{(wwtSG)}$ /day for dugong and green turtle respectively.

	Dugongs	Turtles
Max OA daily intake (ng/day)	18,400	920
Mean OA daily intake (ng/day)	2,440	120
Max OA dose per day per body weight (ng/day/kg)	46	15
Mean OA dose per day per body weight (ng/day/kg)	6	2

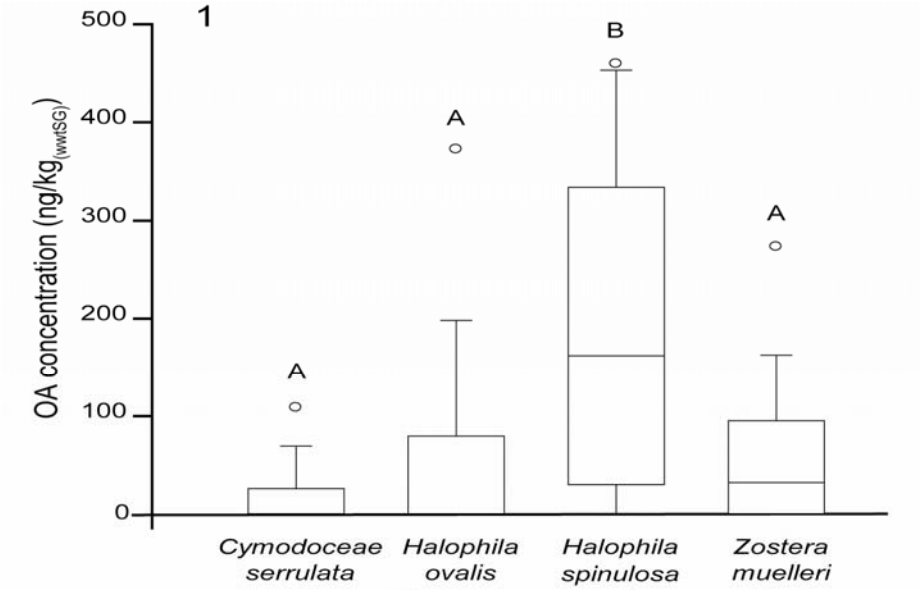

N
0
6
12
Kilometres
Moreton Bay
3
2
1
North Stradbroke Island
Pacific Ocean
Australia
Sampling sites:
1 = Myora Light
2 = Palmer Passage
3 = Rous Channel

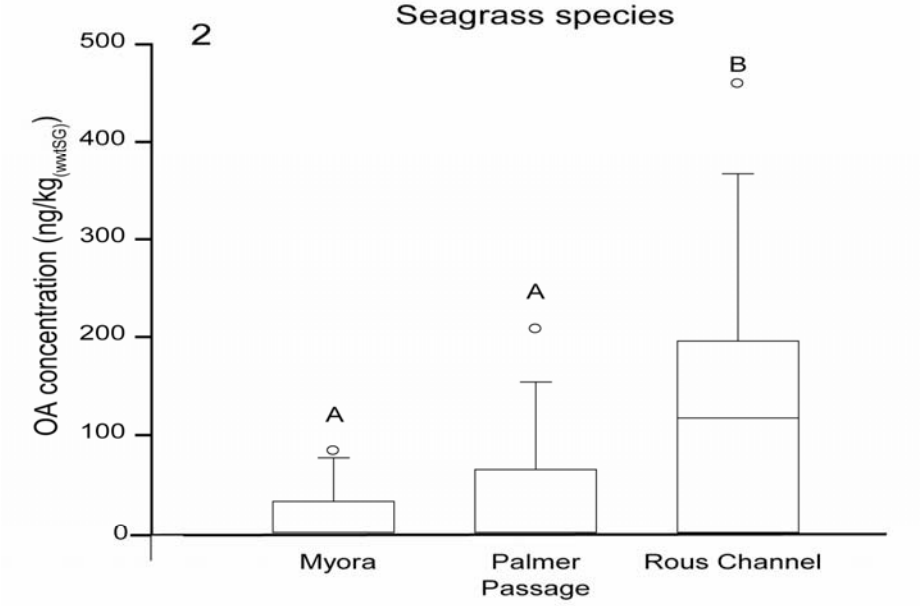
537

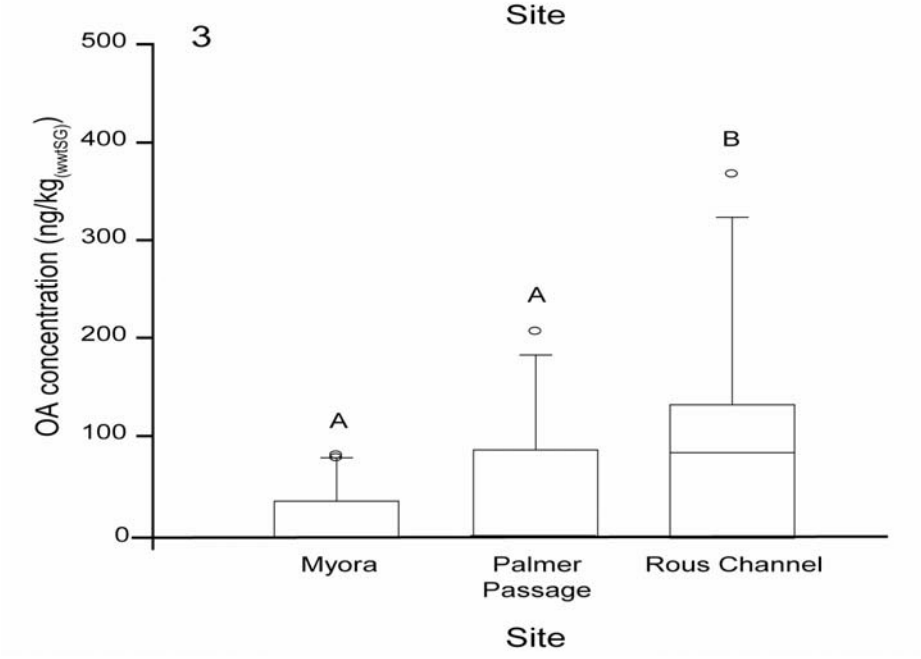
538 Fig. 1. Map of seagrass sampling sites around North Stradbroke Island, off south-
539 eastern cost of Australia: 1 = Myora light, 2 = Palmer Passage, 3= Rous Channel.


500
400
300
200
100
0
OA (ng/kg(wwtSG))
0
5
10
15
20
P. lima Abundance (10^5 cells/kg(wwtSG))
r = 0.40
p < 0.0001

543 Fig 2: The relationship between Prorocentrum lima cell abundance and okadaic acid
544 (OA) concentration in epiphyte material collected from seagrass samples collected in
545 Moreton Bay, Australia (n = 99).

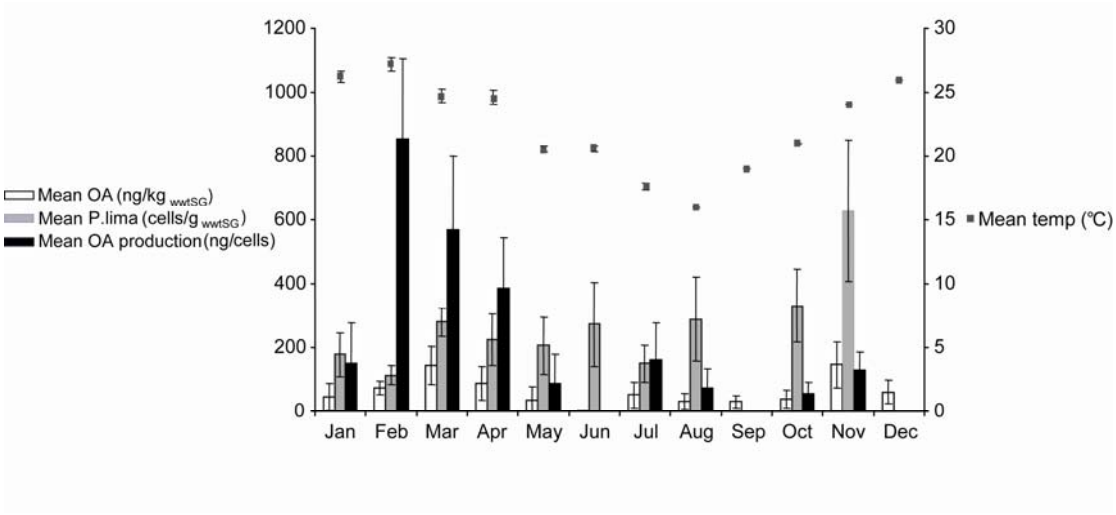

1
500
400
300
200
100
0
OA concentration (ng/kg(wwtSG))
A
A
B
A
Cymodoceae serrulata
Halophila ovalis
Halophila spinulosa
Zostera muelleri
Seagrass species


2
500
400
300
200
100
0
OA concentration (ng/kg(wwtSG))
A
A
B
Myora
Palmer Passage
Rous Channel
Site


3
500
400
300
200
100
0
OA concentration (ng/kg(wwtSG))
A
A
B
Myora
Palmer Passage
Rous Channel
Site

546

Fig. 3. Box plots showing the concentration of okadaic acid found in epiphyte material collected from 1. four species of seagrass with all sites combined, 2. three sites in Moreton Bay, Australia combining all seagrass species and 3. combined data for Z. muelleri and H. ovalis at all sites. The bottom and top of the box represents the 25^{th} and 75^{th} percentile respectively, while the median is represented by the bar in the middle of the box. Error bars represent 90^{th} percentile and dots the maximal value observed. Statistically significant groups (Dunn's post hoc multiple comparisons test) are denoted by the letter above the box.


Mean OA (ng/kg wwtSG)
Mean P.lima (cells/g wwtSG)
Mean OA production (ng/cells)
Mean temp (°C)
1200
1000
800
600
400
200
0
30
25
20
15
10
5
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

555

Fig. 4. Mean OA concentration, abundance of P. lima and temperature over a twelve month period. Error bars represent standard error per month (n = 99).