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# A bloom of *Lyngbya majuscula* in Shoalwater Bay, Queensland, Australia: An important feeding ground for the green turtle (*Chelonia mydas*)

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**Abstract**

*Lyngbya majuscula*, a toxic cyanobacterium, was observed blooming during June–July (winter) 2002 in Shoalwater Bay, Queensland, Australia, an important feeding area for a large population of green turtles (*Chelonia mydas*). The bloom was mapped and extensive mats of *L. majuscula* were observed overgrowing seagrass beds along at least 18 km of coast, and covering a surface area of more than 11 $km^2$. Higher than average rainfall preceded the bloom and high water temperatures in the preceding summer may have contributed to the bloom. In bloom samples, lyngbyatoxin A (LA) was found to be present in low concentration (26 $\mu g\ kg^{-1}{}_{(dry\ weight)}$), but debromoaplysiatoxin (DAT) was not detected. The diet of 46 green turtles was assessed during the bloom and *L. majuscula* was found in 51% of the samples, however, overall it contributed only 2% of the animals' diets. *L. majuscula* contribution to turtle diet was found to increase as the availability of the cyanobacterium increased. The bloom appeared to have no immediate impact on turtle body condition, however, the presence of a greater proportion of damaged seagrass leaves in diet in conjunction with decreases in plasma concentrations of sodium and glucose could suggest that the turtles may have been exposed to a substandard diet as a result of the bloom. This is the first confirmed report of *L. majuscula* blooming in winter in Shoalwater Bay, Queensland, Australia and demonstrates that turtles consume the toxic cyanobacterium in the wild, and that they are potentially exposed to tumour promoting compounds produced by this organism.


*Keywords:* *Chelonia mydas*; Cyanobacteria; Diet; Green turtle; *Lyngbya majuscula*; Shoalwater Bay

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## 1. Introduction

Harmful algal blooms (HABs) have been most extensively studied in cases where acute (human) poisoning occurs or where secondary metabolites produced by the blooming species may provide novel compounds with application in the biopharmaceutical industry (e.g., Bonnard et al., 1997; Orjala and Gerwick, 1997; Márquez et al., 1998; Singh et al., 1999; Milligan et al., 2000; Mitchell et al., 2000). The ecological impacts of HABs and the fate of the toxins they produce are not well understood, especially in terms of their implications for wildlife. The effects of HAB toxins and the potential for bioaccumulation of these toxins through the trophic food web are still largely unknown, and the effects of chronic exposure to these compounds is even less understood (Landsberg, 2002).

In recent years, an increase in the frequency and severity of *Lyngbya majuscula* blooms in Queensland, Australia (Dennison et al., 1999) has led to concern for their impacts on local fauna. *L. majuscula* is a cyanobacterium that is found worldwide in tropical and subtropical estuarine and coastal habitats (for review, see Osborne et al., 2001). It grows epiphytically on seagrass, macroalgae, rock, coral and anthropogenic structures forming matted masses of dark filamentous material (Humm and Wicks, 1980; Jones, 1990; Dennison et al., 1999). Previously known as *Microcoleus lyngbyaceus* (Kützing) and locally known as 'mermaid hair' or 'blanket weed', it is usually dark olive in appearance, but, it has also been reported as being red, black, brown and white in colour (Banner, 1959; Izumi and Moore, 1987; Dennison et al., 1999; Osborne et al., 2001). It is generally found in the inter-tidal and sub-tidal habitat (Osborne et al., 2001), although it has been observed as deep as 30 m (Izumi and Moore, 1987). Blooms in Australia have been described in Deception Bay, Moreton Bay, Fraser Island and Hardy Reef and have been observed to continue for up to 4 months (Dennison et al., 1999; Albert et al., 2005).

*L. majuscula* is a prolific producer of secondary metabolites, with over 70 compounds produced by the cyanobacteria found to have biological activity (see reviews by Nagle and Paul, 1999; Burja et al., 2001; Osborne et al., 2001). These include tumour promoters such as lyngbyatoxin A (LA) and debromoaplysiatoxin (DAT) (Cardellina et al., 1979; Fujiki et al., 1984a), immunosuppressants (Koehn et al., 1992), cytotoxic compounds (Todd and Gerwick, 1995; Harrigan et al., 1998; Luesch et al., 2002), herbivory antifeedants (Nagle et al., 1996) and antineoplastic compounds (Mynderse et al., 1977). Blooms of *L. majuscula* have caused contact dermatitis and breathing difficulties in humans (Grauer and Arnold, 1961; Fujiki et al., 1985; Osborne et al., 2001) and are believed to be the causative agent in a number of human food poisonings where the cyanobacteria was incidentally ingested along with edible seaweed (Cardellina et al., 1979; Sims and Zandee Van Rilland, 1981; Hanne et al., 1995; Nagai et al., 1996). In one case, consumption of turtle meat in Madagascar led to a fatality in which LA was determined to be the causative agent (Yasumoto, 1998). Ito et al. (2002) demonstrated that oral ingestion of LA by mice resulted in injuries to the stomach, intestines, lungs and liver, while less specific studies showed that mice administered a water-soluble extract from *Lyngbya* spp. demonstrated a cessation in feeding, weight loss, decreased immunocompetancy (Sundararaman et al., 1994) and changes in blood biochemistry (Sundararaman et al., 1996). Lyngbyatoxin A and DAT are known to cause human contact dermatitis (Fujiki et al., 1984a,b) and these compounds have also have been demonstrated to be potent tumour promoters in mice (Fujiki et al., 1984a, 1985).

Ingestion of naturally produced tumour promoting compounds by green turtles has been implicated in the aetiology of the debilitating marine turtle disease fibropapillomatosis (FP) (Landsberg et al., 1999). Fibropapillomatosis occurs worldwide in epizootic levels, but is in greatest prevalence in inshore regions of high human impact. The disease manifests itself as raised tumourous masses, usually found on the eyes, soft skin of the inguinal region, mouth or internal organs. The growths are benign, but can lead to death through reduced mobility and loss of vision (Herbst, 1994). The cause of the disease is unknown, however, it has been associated with a herpes virus (Jacobson et al., 1991; Lu et al., 2000; Quackenbush et al., 2001) and a role for naturally produced tumour promoters such as LA and DAT has also been suggested (Landsberg et al., 1999).

This study describes a bloom of *L. majuscula* that occurred in Shoalwater Bay, Central Queensland,

Australia during June/July (winter) 2002. The bloom occurred in a restricted access area within the inter-tidal waters of Queensland and adjacent to the Great Barrier Reef Marine Park in a region bordering a military training area. Shoalwater Bay is a shallow embayment that is subject to a large tidal range (7 m during spring tides). The bay is fringed by mangroves and extensive inter-tidal and sub-tidal seagrass beds (Commonwealth of Australia, 1994; Long et al., 1997) that provide an important foraging area for a large population of green turtle (*Chelonia mydas*) (Limpus et al., 2001, 2002, 2003) that is the subject of this study.

Green turtles are marine reptiles that, when young (5–10 years old), take up residency in a feeding area to which they show high site fidelity over decades (Chaloupka et al., 2004; Limpus, 2004). They are primarily herbivorous, feeding on seagrass and/or macroalgae (Garnett et al., 1985; Forbes, 1995; Read and Limpus, 2002) and as their diets have also been shown to include animal material where available (Hirth, 1971; Bjorndal, 1997; Read and Limpus, 2002) and mangrove fruit when in season (Pritchard, 1971; Pendoley and Fitzpatrick, 1999; Limpus and Limpus, 2000; Read and Limpus, 2002), they also appear to be opportunist feeders. The volume that both these items contribute to diet, and the change in feeding behaviour required to consume them, suggests that the turtles are demonstrating a level of selectivity and not merely incidentally ingesting items that are found on or near their regular food (Read and Limpus, 2002). It is not known whether turtles would selectively feed on, or try to avoid a cyanobacterium known to produce secondary metabolites that act as a feeding deterrent to other herbivores (Nagle and Paul, 1999; Capper, 2004).

Turtles in captivity have been observed to ingest *L. majuscula* (McMaster, 2002) and it has been found in the gut contents of marine turtles from the Hawaiian Islands (Russell and Balazs, 2000). It is not known whether this is selective preference or incidental ingestion, but it does demonstrate the potential for wild marine reptiles to be exposed to cyanotoxins. In Shoalwater Bay, the seagrasses *Zostera muelleri*, formerly *Z. capricorni* (Les et al., 2002), *Halophila ovalis* and *Halodule* spp. are the dominant benthic vegetation (Lee Long et al., 1996) and green turtles have been observed feeding on *Halophila* spp. and *Halodule* spp. (Limpus and Limpus, 2000). When in bloom, *L. majuscula* grows on a variety of benthic substrates, including seagrass and macroalgae (Dennison et al., 1999). We suggest that when *L. majuscula* is in abundance, such as under bloom conditions, the turtles ingest the cyanobacterium whilst feeding on the seagrass to which the *L. majuscula* is attached.

To assess the impact of this *L. majuscula* bloom on green turtles in Shoalwater Bay, we characterised the bloom in terms of coverage, extent and toxin production and assessed the exposure of green turtles to toxins produced by the cyanobacteria. As the most common route of exposure to HAB toxins is through ingestion (Landsberg, 2002), and as green turtles are herbivorous, we investigated the diet of green turtles during the bloom. As marine turtles may not feed for up to 3 months while on breeding migrations (Bjorndal, 1985), it was important to assess whether turtles cease feeding during a toxic algal bloom or, if they continue to feed, whether they consume the cyanobacteria either actively or incidentally as part of their normal diet. Behavioural observations of the turtles were made during the bloom and the impacts of the bloom were considered by comparing blood biochemistry and turtle body condition between turtles captured in 2002 and turtles that were captured the following year when no *L. majuscula* was observed in the feeding ground.

## 2. Methods

Shoalwater Bay (22°20S, 150°12E) is a shallow embayment on the Central Queensland Coast of Australia (Fig. 1). The catchment is largely unaltered due to the presence of the military training area (reserved since 1965), however, historically the area was used for grazing, wood harvesting and at one time gold was extracted (Gunn et al., 1972). Currently, there is little human disturbance in the catchment with minimal development and no coastal towns (Long et al., 1997). The area has variable and generally low annual rainfall (800–1600 mm) and many of surface streams run dry for much of the year (Commonwealth of Australia, 1994).

The bloom of *L. majuscula* was observed in Shoalwater Bay over a 2-week period from 22 June to 5 July 2002. The extent of the bloom was mapped

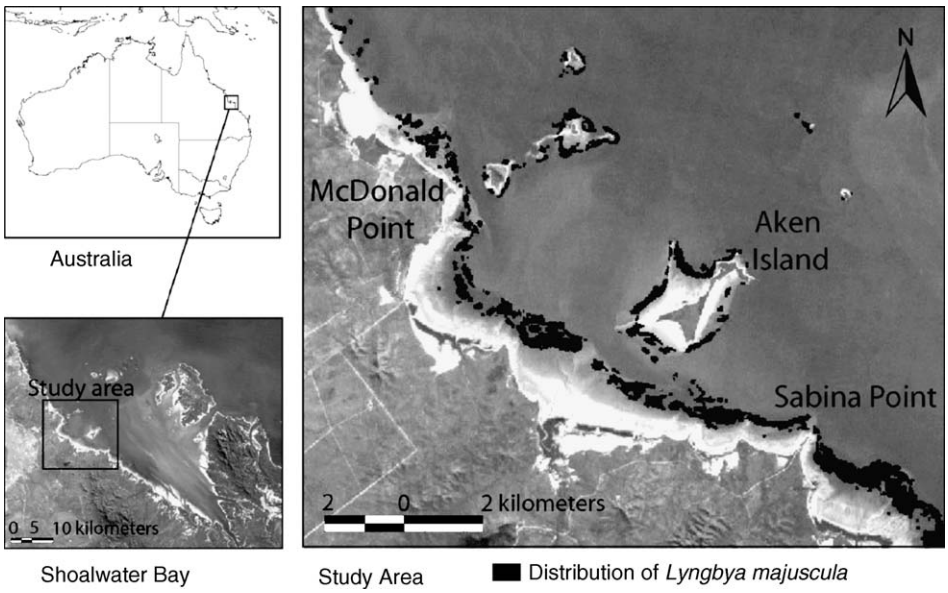


Fig. 1. Characterised Landsat 7 ETM image (9:45 a.m. 29 June 2002) of Shoalwater Bay with *Lyngbya majuscula* bloom distribution map overlayed. Line map of Australia shows Shoalwater Bay on the Central Coast of Queensland and the regional view of the Landsat image shows the location of the study site within the Western Bight of Shoalwater Bay. The bloom was observed along 18 km of coastline and found to cover 11 $km^2$ of inter-tidal seagrass habitat.

using a combination of field data and satellite imagery (Landsat 7 Enhanced Thematic Mapper ETM) after the methods of Roelfsema et al. (2002). This involved assessing the percent cover of *L. majuscula* and seagrass at 150 points across the bay. Assessments were made either by walking transects at low tide or by surface observation from a boat. Where poor water clarity impeded observations, assessments were made on snorkel. Percent cover was estimated at each site (none = 0%, sparse = 1–10%, low = 11–40%, medium = 41–70%, high > 70% cover). Sites were haphazardly selected throughout the bloom for assessment, and edge and non-bloom points were specifically selected to allow geo-referencing of the satellite image. Geographic coordinates (reference datum WGS84) were determined for each site, using a hand-held Garmin 12 Global Positioning System (GPS) (Garmin Inc., Kansas City). Using this percent cover data, a supervised classification was conducted of a Landsat 7 ETM image. The image was acquired on 29th June 2002, coinciding with the week of field data collection. Supervised classification involves establishing an image library of spectral signatures for all benthic substrates (e.g. *L. majuscula*) (Lillesand and Kiefer, 2000). This library is then used to identify all pixels in the image with a similar spectral signature, which then results in an image presenting the distribution of each benthic cover type. A *L. majuscula* distribution map was then produced using Geographic Information System (GIS) software, ArcView GIS 3.1 (Environmental Systems Research Institute, USA) and used to calculate surface area covered by *L. majuscula* and non-*L. majuscula* (e.g. seagrass and sand) for the study area (Fig. 1). The distribution map was also divided into eight sectors, the boundary of which was determined as the region in each sector where seagrass could potentially grow. This analysis resulted in an estimate of *L. majuscula* coverage for each sector which was used as a proxy of availability.

To confirm the identification of the cyanobacteria, a voucher sample of the cyanobacteria was collected, stored in 10% formalin and identified by Dr. Julie Phillips (University of Queensland). Three additional samples of *L. majuscula* were collected for detection of LA and DAT. These samples were collected from areas where turtles were observed feeding and were growing epiphytically on seagrass. They were

immediately frozen and returned to laboratory for analysis. To extract toxins the samples were freeze-dried, ground and 1 $g_{(dry\ weight)}$ extracted overnight using acetone (AnalaR, Merck Pty, Victoria, Australia). Samples were sonicated (Branson Sonifer 250) and vacuum filtered using Whatman glass microfibre filters. Acetone was removed via rotary evaporation (Bücher Rotary Evaporator, Switzerland) and the extract resolubilised in 50% methanol (Mallinckrodt ChromAR HPLC, Kentucky, USA). The concentration of LA and DAT were determined by HPLC–MS/MS using PE/Sciex API 300 mass spectrometer equipped with a high flow electrospray interface (TurboIonspray) coupled to a Perkin-Elmer series 200 HPLC system. Separation was achieved using a 150 mm × 4.6 mm Altima $C_{18}$ column (Alltech) run at 35 °C, with mobile phase consisting of 80/20 acetonitrile/hi-pure water containing 0.1% formic acid and 2 nM ammonium formate at a flow rate of 0.8 ml $min^{-1}$. The flow was split post column such that the flow to the mass spectrometer interface was 250 μl $min^{-1}$. Under these conditions the retention times were 11.72 and 9.3 min for LA and DAT, respectively. The mass spectrometer was operated in the positive ion, multiple ion-monitoring mode. Ions monitored with dwell times of 300 ms were 410.3 and 438.3 $(M + H)^+$ for LA and 543.3 for DAT. Quantification was achieved by comparison to standard solutions of LA (Calbiochem, Lot #B41940) and DAT generously provided by Dr. R.E. Moore (University of Hawaii) run under the same conditions. Using a 20 μl injection the detection limit for both toxins was typically 0.03 mg $kg^{-1}{}_{(dry\ weight\ cyanobacterium)}$.

Sub-tidal water temperature was measured during the sampling period using a Minilog data logger (Vemco™, Nova Scotia) that was not exposed at low tide with a minimum depth of ∼1.5 m. The water temperature for the lower inter-tidal region for the 10 months preceding the bloom was recorded using the same data logger placed in an inter-tidal pool on the sand flats. Rainfall data was generously provided by the Australian Department of Defence, Shoalwater Bay Training area. Rainfall was measured for Pine Mountain and data were provided as monthly totals from January 1988 to June 2003.

Green turtles were captured as part of a long-term population demography study of marine turtles in Shoalwater Bay using the turtle rodeo technique (Limpus and Reed, 1985). The turtles were either tagged immediately or, in the case of recaptured turtles, previously applied tags were recorded along with a GPS location for the first sighting of the animal. Turtles were returned to base where the curved carapace length (CCL) was measured using a flexible tape measure and their weight measured to the closest 0.5 kg using a Salter scale. The sex and maturity of each turtle was determined using laparoscopy techniques and turtles were classified as immature, pubescent or adult based on gonad maturation (Limpus et al., 1994). Each turtle was also assessed for the presence of fibropapilloma lesions and severity of the disease was quantified as per Work and Balazs (1999). Turtles were considered to be in regression when the severity of lesions declined, or when no lesions were observed on an animal that had previously been observed with tumours (Herbst, 1994).

A sub-set of turtles was selected for diet analysis. Food from the most recent feeding event was obtained from the lower oesophagus/crop using the stomach flush technique (Forbes and Limpus, 1993) and the content and relative volume of material identified from crop samples was determined using the principles of microstereology (Schaefer, 1970) after the modified methods of Forbes (1995) and Read (1991). The sample was viewed under a dissection microscope (×7) and all material was visually identified. Seagrass was identified using Channells (1981) and Lanyon (1986) and algae using Cribb (1983, 1996) and Huisman (2000). To quantify the sample, an eye-piece graticule with 33 marked positions was used to determine the volumetric proportion of each food item by counting the number points covering each food type. Ten non-overlapping fields of view were observed and a total of 330 points used for each sample. The relative proportion of each food type present was found by dividing the number of points covering each food type by the total number of points observed and then multiplied by 100 to give a percentage of diet.

In addition to the identification of the seagrass, the condition of each seagrass blade in all diet samples was assessed and quantified. A subjective measure of health was made based on colour of the leaf blade and continuity of the leaf edge. Seagrass blades were considered to be ‘‘healthy’’ when they appeared bright green in colour with distinct sharp edges (Fig. 4) and

''damaged'' when dark brown to black and disintegrating around the edge as described for thermally stressed seagrass (Walker and Cambridge, 1995). The proportion of ''damaged'' seagrass leaves in diet was calculated as per other dietary items. To determine whether there was an unusually high proportion of ''damaged'' seagrass leaves in green turtle diet samples during the bloom event, diet samples from 2002 were compared with diet samples collected in an identical manner during 2003 when no bloom of *L. majuscula* was present in Shoalwater Bay. The field trip to Shoalwater Bay in 2003 was conducted at the same time of year (28 June–11 July) and transects of the bay were run to assess the presence of *L. majuscula*. As no *L. majuscula* was observed, diet analysis, blood biochemistry and body condition index were compared between the two sampling events to assess the impacts of the bloom on the green turtle population.

Blood samples were collected from turtles as per Owens and Ruiz (1980). Approximately 5 ml of blood was obtained from the dorsal cervical sinus, normally within 3–6 h of capture. The blood was centrifuged at ~5000 rpm for 5 min and the plasma separated and frozen. Plasma concentrations of calcium, magnesium, sodium, lactate dehydrogenase (LDH), cholesterol, triglycerides and glucose were measured by the University of Queensland Department of Veterinary Pathology Unit using an automated Olympus AU400 analyser and Olympus reagents (Olympus Diagnostica, Clare Island). As plasma biochemistry values in marine turtles have been shown to be effected by disease (Aguirre et al., 1995; Aguirre and Balazs, 2000; Swimmer, 2000), we used only turtles that were considered clinically healthy (i.e. no external lesions, injuries or signs of poor condition) for comparative analysis.

Body Condition Index (BCI) is often used as an indicator of animal health. In this study, BCI was defined as the residual of the regression of turtle mass (kg) on curved carapace length (cm) (Jessop et al., 2004). To ensure the most accurate assessment of BCI, all turtles that were captured in each sampling year were considered ($n = 756$). Turtle mass ranged between 6.0 and 177.0 kg and mass = $0.00009 \times \mathrm{CCL}^{3.0547}$ (Fig. 5). A positive residual (BCI) indicated an above average weight for length, while a negative residual indicated a below average weight.

All analyses were performed using Statistica V6.1 (StatSoft Inc., Oklahoma). Turtle diet data was assessed for homogeneity of variance using Brown and Forsyth test and the effects of sex and age on the proportion of *L. majuscula* in turtle diets were tested using a *t*-test independent by groups and one-way analysis of variance (ANOVA) respectively. The relationship between *L. majuscula* coverage in each sector and the amount *L. majuscula* contributed to the diet in turtles captured in that sector was compared using a Pearson product-moment linear correlation. The ratio of healthy to damage seagrass leaves was transformed using a natural log transformation to ensure the data fitted the assumption of parametric tests and the difference between years was assessed using a *t*-test grouped independently by year. Three-way multivariate analysis of variance (MANOVA) was used to test the effects of age, sex and the presence of a *L. majuscula* bloom on the blood biochemistry of the turtles. As all blood biochemical parameters were measured for each turtle, the parameters were not independent and therefore MANOVA was deemed to be an appropriate test. However, as changes in each biochemical parameter was of interest, a three-way analysis of variance (ANOVA) was also used to compare the impacts of age, sex and the presence of a bloom on each biochemical parameter. Where a significant result was obtained for the three-way ANOVA, individual means were compared using an unequal *n* Tukey's post hoc test. Triglyceride, LDH and glucose data were log transformed to satisfy assumptions for ANOVA. Body condition of turtles captured during the bloom was compared with the condition of turtles captured the following year when no bloom was present using a one-way ANOVA of BCI (residuals of mass on length). Significance was accepted at $\alpha = 0.05$ and all results are presented as means ± standard error (S.E.).

## 3. Results

This is the first time a bloom of *L. majuscula* has been documented in Shoalwater Bay. The bloom was present during the entire study period from 22 June to 5 July (winter) 2002 and was found to extend along at least 18 km of coastline. The *L. majuscula* distribution map indicated that the bloom covered at least 11 $\mathrm{km}^2$

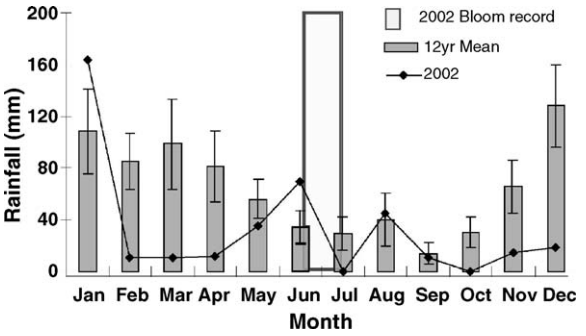


Fig. 2. Shoalwater Bay rainfall data recorded at Pine Mountain (data supplied by Australian Defence Force) showing 12-year mean and monthly average for 2002. Light shaded area during June–July depicts the observed presence of *Lyngbya majuscula* bloom during 2002.

of the study area (Fig. 1). The spectral image suggests that the bloom extended beyond the surveyed region, however, this area could not be quantified with confidence due to the lack of ground truthed data outside the study area. The overall accuracy (Lillesand and Kiefer, 2000) of the image classification resulting in the distribution map was 60%. It is likely that the bloom covered a much greater area than reported in the current study since the image classification is less reliable at depth where *L. majuscula* cannot be optically identified. *L. majuscula* was found both through field observations and image analysis to be predominantly in the lower inter-tidal and sub-tidal zones (to about 2 m in depth at low tide). The duration of the bloom is unknown as it was present when researchers arrived and still evident when researchers left 14 days later. A subsequent visit to the region by co-workers in August of the same year found no *L. majuscula* present at the site (Dr. James Udy, 2002, personal communication).

During the months preceding the bloom there had been an extremely hot summer, with higher than average rainfall (Fig. 2) and abnormally high water temperatures on the inter-tidal banks. During January and February water temperatures in tidal pools reached over 35 °C regularly and coral bleaching was observed in adjacent reefs. Although the January average rainfall was above the 12-year monthly average, the 3 months preceding the bloom had below average rainfall, except for an event just prior to the start of the study period (Fig. 2). Water temperatures in the inter-tidal zone during the bloom were 16.6–21.4 °C.

Although working amongst the *L. majuscula* for two weeks, only one researcher noted skin irritation with reddening of skin where clothing was in close contact. The concentration of the dermatitis producing agents and tumour promoting compounds LA and DAT were found to be negligible with no DAT detected in any of three samples and only one sample was found

Table 1
The sex and age class of green turtles sampled for dietary analysis and blood biochemistry analysis in Shoalwater Bay during the 2002 bloom of *Lyngbya majuscula* and a comparative group captured during 2003 when no *L. majuscula* was present in the feeding ground

| Year (total no. of turtles captured) | Sample group | Sex | Maturity status | | | Total |
|---|---|---|---|---|---|---|
| | | | Immature | Pubescent | Adult | |
| 2002 *L. majuscula* bloom (410) | Diet analysis | Female | 16 | 8 | 11 | |
| | | Male | 9 | 0 | 2 | |
| | | Total | 25 | 8 | 13 | 46 |
| | Blood samples | Female | 7 | 6 | 1 | |
| | | Male | 4 | 0 | 2 | |
| | | Total | 11 | 6 | 3 | 20 |
| 2003 no bloom (346) | Diet analysis | Female | 22 | 3 | 11 | |
| | | Male | 6 | 2 | 3 | |
| | | Total | 28 | 5 | 14 | 47 |
| | Blood samples | Female | 15 | 3 | 5 | |
| | | Male | 6 | 1 | 2 | |
| | | Total | 21 | 4 | 7 | 32 |

The number in the brackets under year represents the total number of turtles captured during the field trip. The turtles sampled for blood biochemistry analysis are a sub-sample of the group selected for diet analysis.

to contain a detectable concentration of LA at 0.26 mg $kg^{-1}_{(dry\ weight\ cyanobacteria)}$.

During the 2002 study period 417 green turtles were captured in the region of the bloom. Fibropapilloma prevalence amongst these turtles was low with only 1.2% of captured turtles expressing external lesions and 0.7% of turtles captured during 2002 had previously been observed with tumours and were in regression. Of the 417 turtles captured during 2002, 46 were stomach flushed and the sex and age class of these animals is outlined in Table 1. The turtles were found to be feeding primarily on the seagrasses *Zostera muelleri* and *Halodule* spp. Red algae and animal material also contributed to diet (Fig. 3A). *L. majuscula* was common in green turtle diet samples as 51% of samples contained some *L. majuscula* (Fig. 3A). However, as a proportion of diet, *L. majuscula* contributed only 1.6% of the mean relative volume (Fig. 3B). Even when only animals that were found to have *L. majuscula* in their diet sample were considered ($n = 24$), it still contributed an average relative volume of only 3.2% with a maximum observation of 13.6% of diet, suggesting that *L. majuscula* does not contribute greatly to green turtle diets even when readily available. Neither the sex nor age class of the turtle was found to impact on the contribution of *L. majuscula* in the turtles' diet (sex: *t*-test: $F = 1.67$, d.f. = 42, $P = 0.41$; age-class: one-way ANOVA: $F = 0.543$, d.f. = 3, $P = 0.655$). However, a positive correlation was found between the availability of *L. majuscula* in the sector in which the turtle was

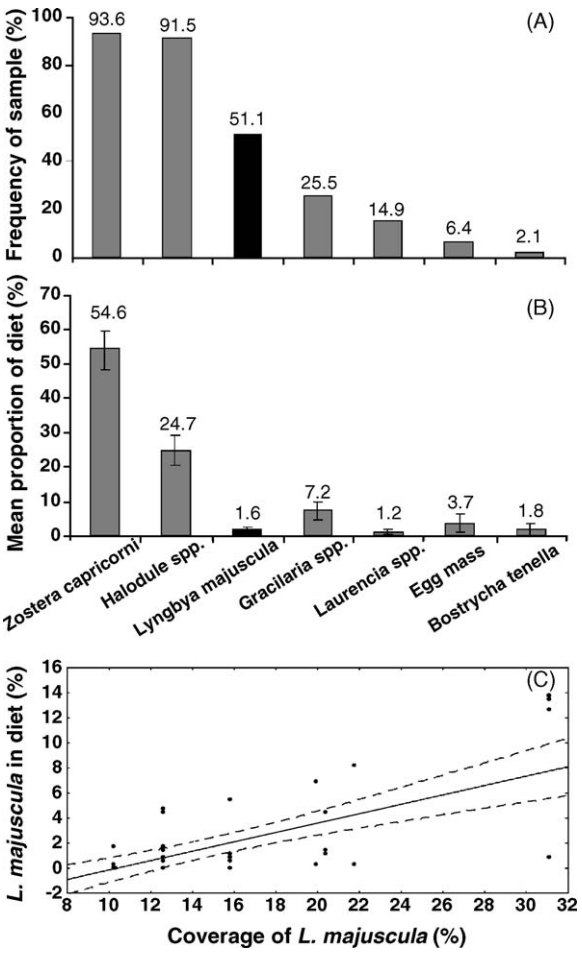


Fig. 3. Diet of green turtles from Shoalwater Bay, June 2002 ($n = 46$). *Lyngbya majuscula* is noted in black. (A) The proportion of all green turtle diet samples containing each food type and (B) the mean relative volume of each food type. (C) Scatter plot showing the correlation between the availability of *L. majuscula* in each sector and the amount that the cyanobacteria contributed to the diet of turtles ($p < 0.001$). The solid line illustrates the correlation ($r^2 = 0.671$) and hatched lines represent the 95% confidence interval. Linear relationship is *L. majuscula* in diet (%) = $-3.91 + 0.38 \times$ *L. majuscula* coverage (%).

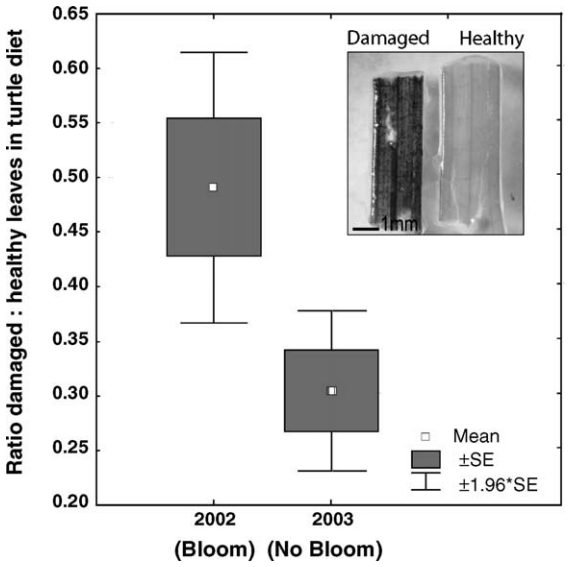


Fig. 4. The ratio of "damaged" to "healthy" seagrass leaves found in green turtle diets in 2002 and 2003 at Shoalwater Bay. Inset shows the difference between "healthy" and "damaged" *Zostera muelleri* leaves found in diet samples. Ratios were significantly different between years (*t*-test on log transformed data: *t*-value = 2.75, d.f. = 93, $P = 0.007$).

Table 2
Univariate analysis of variance for each blood biochemical parameter collected from 52 green turtles during 2002 and 2003 in Shoalwater Bay, Australia

| | Blood biochemistry parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sodium | Calcium | Magnesium | Cholesterol | Triglycerides | Glucose | LDH |
| Lyngbya | <**0.001** | 0.555 | 0.421 | 0.321 | 0.621 | 0.299 | 0.415 |
| Sex | 0.616 | 0.263 | 0.719 | 0.383 | 0.258 | 0.446 | 0.450 |
| Age | **0.012** | <**0.001** | 0.170 | <**0.001** | <**0.001** | 0.814 | 0.218 |
| Lyngbya × sex | 0.606 | 0.986 | 0.716 | 0.940 | 0.405 | 0.202 | 0.851 |
| Lyngbya × age | 0.123 | 0.548 | 0.464 | 0.807 | 0.764 | 0.268 | 0.065 |
| Sex × age | 0.893 | 0.301 | 0.481 | 0.642 | 0.850 | 0.168 | 0.461 |
| Lyngbya × sex × age | – | – | – | – | – | – | – |

captured and the amount of *L. majuscula* in their diet (Fig. 3C).

Turtles also appeared to be avoiding areas most heavily impacted by the *L. majuscula* bloom. From behavioural observations, it appeared that at low tide the turtles were remaining in the deeper water below the bloom and then at high tide swimming up and over the bloom to feed on seagrass in the shallow inter-tidal areas. The high proportion of ''damaged'' seagrass leaves in their diet supported these behavioural observations as damaged leaves contributed an average of 22.0% (±2.6%) of all the seagrass in diet samples. The ratio of healthy to damaged seagrass leaves in turtle diet in 2002 was found to be significantly different from the ratio observed in 2003 (*t*-test on log transformed data: *t*-value = 2.75, d.f. = 93, $P = 0.007$; Fig. 4) with a greater proportion of damaged leaves found in 2002.

Blood samples were obtained from twenty clinically healthy green turtles captured during the 2002 *L. majuscula* bloom. These were compared with blood samples from 32 turtles captured in 2003 when no bloom of *L. majuscula* was present in Shoalwater Bay. The sex and age class of these animals is outlined in Table 1. A three-way MANOVA with age, sex and *L. majuscula* bloom presence as fixed factors and the seven plasma biochemical parameters as dependant variables showed that both age (Wilks' lambda 0.45, $F = 2.49$, $P_{(14, 72)} = 0.006$) and the presence of the bloom (Wilks' lambda 0.48, $F = 5.55$, $P_{(7,36)} = 0.0002$) had a significant effect on turtle plasma biochemistry, but sex did not (Wilks' lambda 0.86, $F = 0.82$, $P_{(7,36)} = 0.57$). Second-degree interactions in the MANOVA were not significant and three-way interactions could not be tested due to an insufficient sample of male pubescent turtles (Table 1). Univariate analysis of variance for each parameter is shown in Table 2. Green turtles captured during the *L. majuscula* bloom were found to have significantly lower plasma concentrations of sodium (142.9 ± 0.8 mmol $l^{-1}$) than those captured the follow-

Table 3
Blood biochemistry values for green turtles captured in Shoalwater Bay

| Parameter | Bloom status | | Age | | |
|---|---|---|---|---|---|
| | Bloom, $n = 20$ | No bloom, $n = 32$ | Immature, $n = 30$ | Pubescent, $n = 10$ | Adult, $n = 12$ |
| Sodium (mmol $l^{-1}$) | 142.9 (0.8) | 151.9 (0.7)* | 150.4 (1.1)[a] | 143.6 (1.0)[b] | 147.7 (1.4)[a] |
| Calcium (mmol $l^{-1}$) | 1.4 (0.1) | 1.1 (0.1) | 1.0 (0.1)[a] | 1.7 (0.1)[b] | 1.4 (0.1)[b] |
| Magnesium (mmol $l^{-1}$) | 3.7 (0.2) | 3.2 (0.1) | 3.1 (0.1) | 3.9 (0.2) | 3.7 (0.2) |
| Cholesterol (mmol $l^{-1}$) | 3.8 (0.3) | 4.2 (0.3) | 3.2 (0.3)[a] | 4.8 (0.4)[b] | 5.6 (0.3)[b] |
| Triglyceride (mmol $l^{-1}$) | 1.4 (0.2) | 1.5 (0.2) | 0.9 (0.1)[a] | 2.2 (0.5)[b] | 2.2 (0.3)[b] |
| Glucose (mmol $l^{-1}$) | 5.1 (0.2) | 6.6 (0.3) | 6.3 (0.3) | 5.1 (0.3) | 6.1 (0.4) |
| LDH (U $l^{-1}$) | 67.1 (9.2) | 61.1 (6.1) | 57.6 (7.1) | 80.3 (12.0) | 63.8 (8.2) |

Values are presented as mean (±SE) for turtles captured during a bloom of *Lyngbya majuscula* (2002) and when no *L. majuscula* was present in the feeding grounds (2003) averaged across age and sex groupings. Mean values for age groupings are averaged across sex and bloom presence groupings. A significant difference ($P < 0.05$) between means of bloom groupings is denoted by (*) and between age groups by (a and b) according to a post hoc unequal *n* Tukey's test.

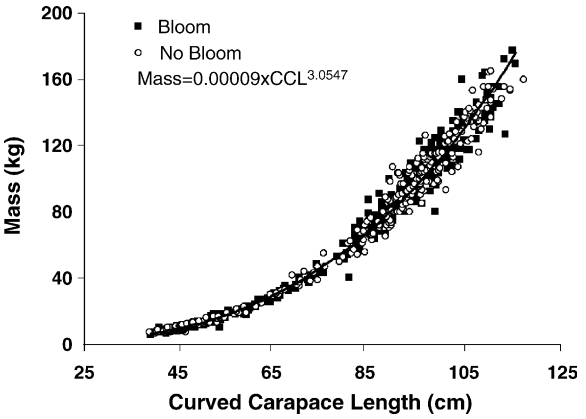


Fig. 5. The relationship between curved carapace length and weight of all turtles captured during a *L. majuscula* bloom (2002) and when no *L. majuscula* was present in the feeding ground (2003). Body condition index for each turtle is defined as the residual of the mass on CCL. There is no significant difference between the BCI for the two samplings (ANOVA: $F = 0.21$, d.f. = 1 $P = 0.65$).

ing year when no *L. majuscula* was present in the feeding ground ($151.9 \pm 0.7$ mmol $l^{-1}$). Although a post hoc test was not employed, glucose concentrations also appeared to be lower in turtles exposed to the *L. majuscula* bloom, while calcium appears to be higher (Table 3). Plasma concentrations of sodium, calcium, cholesterol and triglyceride varied significantly between age groups with immature turtles generally differing from the adult and pubescent turtles (Tables 2 and 3).

Body Condition Index for all green turtles captured in Shoalwater Bay during the *L. majuscula* bloom in 2002 ($n = 410$) was not significantly different from BCI of turtles captured the following year when no bloom was present ($n = 346$) (ANOVA: $F = 0.205$, d.f. = 1, $P = 0.651$; Fig. 5).

## 4. Discussion

An extensive bloom of *L. majuscula* was observed in Shoalwater Bay in the winter of 2002, which may have compromised resource acquisition in the local population of green turtles and potentially exposed them to toxins produced by the cyanobacterium. As marine turtles are able to withstand long periods of aphagia during nesting migrations (Bjorndal, 1985), it was hypothesised that turtles may cease feeding during a large bloom of toxic cyanobacteria such as the one observed in Shoalwater Bay. However, our behavioural observations and the fact that we were able to obtain a diet sample from the anterior crop and/or lower oesophagus suggests that the turtles were continuing to feed during the bloom, albeit in areas where the bloom was least dense.

Although *L. majuscula* was found to be abundant, and was commonly found in diet samples, it only contributed a minor proportion of green turtle diet. This suggests that turtles were attempting to avoid the toxic cyanobacteria and were only incidentally ingesting it when it was attached to the main components of turtle diet. This is supported by evidence that more *L. majuscula* available in the area where the turtles were captured led to a greater proportion of *L. majuscula* in their diet (Fig. 3C). Also, turtles were not observed feeding in areas where the bloom was most dense and instead remained in the deeper channels during low tide and swam past the bloom to the shallow inter-tidal seagrass beds during high tide. It is suggested that this behaviour may have led to the higher proportion of damaged seagrass leaves found in turtle diets during the bloom, as seagrass in the shallow inter-tidal flats becomes brown and desiccated as a result of thermal stress (Walker and Cambridge, 1995; Seddon and Cheshire, 2001). This colour change is not the result of digestion as all samples were recovered from the crop and digestion does not occur in a green turtle crop (Thompson, 1980; Mortimer, 1981). It is hypothesised that the turtles may have been eating this substandard seagrass as the sub-tidal seagrass was covered by *L. majuscula*. Unfortunately, the availability of these leaves in the habitat was not assessed during this study, however, the large presence of browned and desiccated leaves in the diet was noted by the author to be abnormal. The only other published account of dead or damaged seagrass leaves normally found in green turtle diets found that only 7.8% of diet consisted of dead or damage leaved (Mortimer, 1981), which is much lower than the 22% found in this study.

If the high proportion of damaged seagrass leaves represents a sub-optimal diet, then a reduction in body condition would also be expected in turtles that were exposed to the bloom, but this was not the case. There was no significant difference in the body condition of turtles captured during the bloom when compared with turtles from the same population captured the

following year (Fig. 5). This indicates that either the bloom had not been present long enough to cause a detectable loss in weight or the effect of a sub-standard diet was negligible.

Feeding and energy storage has been shown to be the driving factor behind breeding condition in marine turtles and a decrease in the quality or quantity of food availability may lead to decreased breeding rates (Limpus and Nicholls, 1988; Limpus and Chaloupka, 1997) and subsequent population survival (Bjorndal, 1985). During the study a number of adult females that were preparing to breed in the following summer had aborted the process. This relatively uncommon event was also noted in Moreton Bay during a *L. majuscula* bloom in 2000 (Limpus et al., 2002). Currently, the cause of the follicular atresia is not well understood (Hamann et al., 2002), but it is suggested in this case to be indicative of an external stressor either through food limitation as a result of the bloom or through a toxic effect from compounds produced by the cyanobacteria.

The blood biochemistry data from this study also indicates that the turtles demonstrated a physiological response to the *L. majuscula* bloom. Even when variation between age groups was considered, there was still a significant effect of bloom presence on plasma biochemistry and this was driven by a decrease in sodium concentrations in turtles that had been exposed to the bloom (Tables 2 and 3). Hyponatremia can be caused by gastrointestinal loss of sodium as occurs when a reptile suffers from diarrhoea, or it can be related to disorders in salt excretion mechanisms (Campbell, 1996). The reasons for the decrease in sodium concentrations in the current study are not clear and further investigation is required to understand this impact in relation to the cyanobacterium bloom. Although not statistically significant, there was also a decrease in plasma glucose concentrations in turtles exposed to the bloom (Table 3). This may imply that the turtles were not obtaining the required quantity or quality of food as captive green turtles that had been fasting for only a week demonstrated a significant decrease in plasma glucose concentrations (Moon et al., 1999). However, it must be noted that the plasma biochemistry results obtained during this study fall within the ranges previously published for other wild green turtle populations (Bolten and Bjorndal, 1992; Hasbún et al., 1998; Aguirre and Balazs, 2000) and caution should be observed when determining abnormal blood biochemistry in reptiles as their normal healthy ranges can be highly variable (Hasbún et al., 1998).

The concentration of LA and DAT in *L. majuscula* from the Shoalwater Bay bloom was found to be low. No DAT was detected and only 0.26 mg $kg^{-1}$ LA was measured for the bloom. In Moreton Bay, 600 km south of Shoalwater Bay, where blooms of *L. majuscula* have been occurring regularly in recent years, the concentration of these toxins range up to 133 mg $kg^{-1}$ for LA and 23.6 mg $kg^{-1}$ for DAT (Osborne, 2004). Although the toxicity of *L. majuscula* has been found to be highly variable (Banner, 1959; Grauer and Arnold, 1961; Hashimoto et al., 1976; Nagle and Paul, 1999), it should be noted that *L. majuscula* samples collected from Shoalwater Bay in subsequent years yielded similar concentrations of these toxins (Limpus et al., 2003), suggesting that *L. majuscula* from this region may only produce limited amounts of LA and DAT. This may represent a nutrient limitation for the cyanobacterium, as toxin production is likely to be tied to nutrient availability (Osborne, 2004) and the low rainfall in the Shoalwater Bay catchment may lead to lower terrigenous inputs of nutrients.

The presence of LA in the cyanobacterium and the fact that *L. majuscula* was present in turtle diet samples, suggests that the turtles were exposed to the tumour promoter. However, as the duration of this bloom is not known, it is difficult to estimate dose of the toxin in these turtles. If the Shoalwater Bay bloom lasted for only the 2 weeks observed, then an average turtle (weight = 60 kg, eating 218 $g_{(dry\ weight)}$ $day^{-1}$ of seagrass (Bjorndal, 1997)) consuming 2% *L. majuscula* containing 0.26 mg $kg^{-1}{}_{(dry\ weight\ cyanobacteria)}$ would be exposed to 1.1 μg $day^{-1}$ or 15.9 μg LA over the 2 week period. For a 60 kg turtle this is equivalent to 0.26 μg $kg_{(turtle)}{}^{-1}$. Mice (weighing an average of 13 g) given a single oral dose of 1000 μg $kg_{(mouse)}{}^{-1}$ LA did not die, but showed injuries to the lung, stomach, and small intestine (Ito et al., 2002). At the low dose to which turtles in Shoalwater Bay were probably exposed, an acute effect would neither expected, nor was it observed. Changes in blood biochemistry were noted in mice exposed to crude extract from *Lyngbya* sp. (Sundararaman et al., 1996), however, this experiment was non-specific and the

compounds responsible for these changes were not identified. As the LA and DAT concentrations were so low during this bloom, it is likely that the physiological changes observed were related to food limitation rather than to a toxic effect. However, it is recognised that there are many other compounds produced by *L. majuscula* that were not examined in this study (Osborne et al., 2001).

The low production rate of LA and DAT combined with the small amount that *L. majuscula* contributed to diet suggests that the turtles would only be exposed to a small quantity of the tumour promoting compounds in Shoalwater Bay. The fibropapilloma rate observed in this population is also low, with less than 2% of the population observed with tumours. No increase in prevalence of FP has been found in subsequent years (Limpus et al., 2003) and it is suggested that even if the toxins do play a role in the aetiology of this disease (Landsberg et al., 1999), the low concentration found at this site has not impacted on this turtle population.

The cause of blooms of *L. majuscula* in Queensland has not yet been elucidated, however, warm weather, high incident light, enhanced nutrient loading and availability of essential metals from terrigenous sources have been noted to be common factors in algal bloom formation (Paerl, 1988). It has been suggested that blooms in south east Queensland may be related to changes in land-use and are also associated with increased availability of bio-available iron and terrestrial source of organics (Albert et al., 2005; Watkinson et al., 2005). Neither of these hypotheses fit the Shoalwater Bay site as its status as a restricted military reserve means that no land-use changes have occurred in the catchment area since 1965 and the low rainfall in the months preceding the bloom meant that there was little or no terrestrial run-off. The cause of this particular *L. majuscula* bloom is therefore still unknown, but fits the general observation of increased *L. majuscula* observations throughout Queensland.

This study represents the first assessment of the ecological implications of a *L. majuscula* bloom and suggests that the presence of this cyanobacterial bloom may have a negative impact on large marine herbivores such as the green turtle. The blooms possibly hamper the animals' ability to acquire resources for growth and development and may lead to decreased reproductive rates if the blooms increase in frequency. Although this study demonstrates that turtles probably try to avoid the cyanobacteria and probably only consume it incidentally, it does demonstrate that turtles may be exposed to toxins produced by this organism. In Shoalwater Bay, the production of the tumour promoting toxins LA and DAT was low, and therefore the turtles were only exposed to low doses, however, in other areas where toxin production is greater, it is likely that turtles would be exposed to much higher doses.

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