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Potential effects of *Lyngbya majuscula* blooms on benthic invertebrate diversity and shorebird foraging ecology at Roebuck Bay, Western Australia: preliminary results

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Abstract

Nutrient enrichment can significantly alter biodiversity, producing shifts in assemblages of primary producers and favouring, for example, cyanobacterium blooms. These variations in the assemblage of primary producers consequently affect the primary consumers that depend on them. However, the consequences of these blooms for higher trophic levels are still unclear. Roebuck Bay, in the west Kimberley region is one of the main non-breeding areas for migratory shorebirds in Australia. The bay is characterised by an extremely high diversity and biomass of benthic invertebrates, which places this tropical intertidal area among the richest mudflats in the world, and it is likely that this rich benthic fauna supports the shorebird populations. Recent studies in Roebuck Bay have detected nutrient enrichment, with increasing frequency of cyanobacteria blooms. Here we present the preliminary results of the potential effects that *Lyngbya majuscula* (cyanobacterium) blooms have on the benthic invertebrate diversity and shorebird foraging ecology at Roebuck Bay. A site where *Lyngbya majuscula* was present showed a significant diminution in the diversity of benthic invertebrates relative to areas without a bloom. Also, although there was no apparent *Lyngbya*-induced change in the main prey of Bar-tailed Godwits *Limosa lapponica*, there was a change in the foraging behaviour of godwits in the area affected by *Lyngbya*, which appears to relate to a shift in diet. Nevertheless, although we found a correlation between *Lyngbya* presence and shifts in invertebrate assemblages, further work is required to confirm our findings.

Keywords: *Lyngbya majuscula* blooms, benthic invertebrate, shorebirds, Roebuck Bay, Kimberley

Introduction

A major challenge in ecology and conservation research is to improve our understanding of ecosystem diversity and function to develop proper protection, monitoring and management programs that assure their existence for future generations. Coastal ecosystems are heterogeneous, often being characterised by high levels of production (Borges *et al.* 2006) and an immense diversity of ecological processes (Constanza *et al.* 1993). However, coastal habitats are also subjected to high anthropogenic pressure, and as a result they represent the most endangered ecosystems in the world (Duarte 2007). More than one third of the human population lives on the coast and consequently between 30% and 50% of the world's principal coastal areas have been degraded in the last three decades (Duarte 2007). Determination of the effects that human activities have on these ecosystems is of primary importance since overharvesting of marine organisms, land reclamation and more recently, nutrient loading and climate change are pervasively changing coastal wetland ecosystems throughout the world (Vitousek *et al.* 1997; Edgar *et al.* 2000).

Anthropogenic nutrient enrichment of wetlands has become a premier issue for both scientists and managers. Studies of nutrients loads on aquatic systems have determined that nutrient enrichment can significantly alter biodiversity, producing for example shifts in the assemblages of primary producers and favouring phytoplankton, cyanobacterium or macro-algal blooms that are related to episodes of anoxia and hypoxia (Cloern 2001). These variations in the assemblage of primary producers consequently affect the primary consumers that depend on them, often causing them to disappear (Valiella *et al.* 1997; Tewfik *et al.* 2005). However, the consequences of such blooms of primary producers for higher trophic levels are still unclear.

Worldwide there are less than twenty regions where large mudflats rich in shorebirds are found at low tide and only two are located in tropical regions, Roebuck Bay being one of them. Roebuck Bay, located in North-Western Australia is one of the main non-breeding areas for migratory shorebirds of the East Asia-Australasian Flyway (Bamford *et al.* 2008). The importance of Roebuck

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Bay appears to relate to the elevated diversity and biomass of benthic invertebrates (the primary food source of shorebirds), which places this tropical intertidal area among the richest mudflats in the world (Rogers *et al.* 2003). The number of shorebirds using Roebuck Bay may exceed 125,000 in the non-breeding season (Rogers *et al.* 2011), making it one of the most important two shorebird sites in Australia (Rogers *et al.* 2003; Rogers *et al.* 2011). However, 20% of shorebird species that regularly migrate along the East Asian-Australasian Flyway have been officially classified as globally threatened, possibly due to land modification or habitat degradation along the flyway (Rogers *et al.* 2010). Therefore there is an urgent need to monitor and conserve the remaining important sites in the flyway. Roebuck Bay was designated as a Wetland of International Importance in 1990 under the Ramsar Convention (1971), has been recently proposed as a Marine Park, and currently ranks in the top 8 migratory shorebird non-breeding sites in the world (Rogers *et al.* 2003). It is, therefore, a highly significant bird habitat worthy of preservation at a national and international level.

Roebuck Bay is adjacent to the tourist town of Broome (15,857 inhabitants – data for 2009, Australian Bureau of Statistics. Population could double during the tourist season), and recent studies indicate a developing issue with respect to nutrient contamination. A study on regional groundwater has shown elevated nutrient levels in water originating from the area of Broome and moving into the bay (Vogwill 2003). Stable isotope studies have detected elevated $\delta^{15}N$ signature in phytoplankton and filamentous algal from the bay, indicative of nutrient enrichment of the foodweb (A.W. Storey, UWA, unpub. data 2006). A preliminary assessment of the nutrient loads in sediments adjacent to Broome's Town Beach indicate elevated levels of P and N (Pearson 2008) and blooms of cyanobacterium (blue-green algae) *Lyngbya majuscula* first appeared during the 2005 wet season, and have increased in extent each wet season since then (de Goeij *et al.* 2008**;** Pearson 2008).

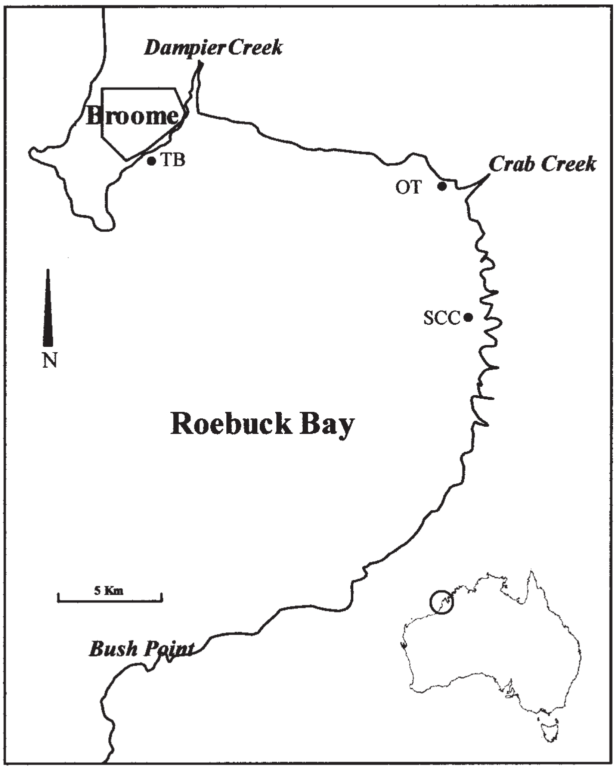


Figure 1. Map of Roebuck Bay, in North Western Australia, with the study sites indicated as TB (Town Beach), OT (One Tree) and SCC (south of Crab Creek).

Here we present the preliminary results of ongoing research looking at the effects that *Lyngbya majuscula* blooms have on the benthic invertebrate diversity and shorebird foraging ecology in Roebuck Bay.

Some described effects of *Lyngbya majuscula* and other cyanobacterium on marine organisms have been attributed to the changes produce in the physicochemical and biological conditions of the habitat (*e.g.* anoxia, covering and smothering of seagrass meadows – *e.g.* Watkinson *et al.* 2005). It is expected then that *Lyngbya* blooms affect the benthic invertebrate abundance (García and Johnstone 2006) and diversity in affected areas of Roebuck Bay. Consequently *Lyngbya* blooms could reduce shorebirds' prey availability. This change of habitat conditions may have long term effects at a population level, since the loss or degradation of migratory shorebirds' habitats anywhere along their flyways is capable of precipitating a decline in their populations (Galbraith *et al.* 2002; Thomas *et al.* 2006).

Methods

Study sites

Roebuck Bay has a tropical climate, with a warm-dry season (May–November), followed by a hot-wet season (December–April). In other areas of Australia, such as Moreton Bay, nutrient inputs together with high temperatures, light conditions and iron availability drive *Lyngbya* blooms (Albert *et al.* 2005; Ahern *et al.* 2008; Johnstone *et al.* 2010). *Lyngbya* blooms occur in the wet season each year in the bay (G. Pearson and A.W. Storey personal observations), which appears to indicate that nutrient inputs, result of rainfall run-off that occurs in the wet season, drive *Lyngbya* blooms together with high water temperatures. In line with this, two sampling programs were carried out, one at the end of the dry season (November 2009) before any bloom was evident, and another mid wet season (February 2010) when *Lyngbya majuscula* was present in the bay.

The study was carried out at three locations. Sites were selected in intertidal areas to characterize: (1) an area potentially affected by wastewater treatment plant/urban activities, presenting a high density of *Lyngbya* throughout the wet season (Town Beach, TB), (2) an area located in the mouth of a tidal creek potentially affected by wet season runoff from pastoral land (One Tree, OT), and (3) an area situated in the proximity of an extensive mangrove area and away from obvious nutrient sources (south of Crab Creek, SCC) (Fig. 1).

Town Beach site is characterized by sandy sediments (Pepping *et al.* 1999; Piersma *et al.* 2006), the existence of seagrass meadows and a narrow fringe of mangroves. It is a zone used by the community as a recreational area. The other two sites, One Tree and south of Crab Creek are characterised by muddy sediments (Pepping *et al.* 1999, Piersma *et al.* 2006) and a wider fringe of mangal. The use of One Tree as recreational area is more restricted and the mud flats south of Crab Creek are seldom visited by humans, as they can only be accessed by boat at high tide.

Benthic invertebrate diversity and abundance

To evaluate whether the diversity and abundance of benthic invertebrates varied between the end of the dry season and mid wet season, when *Lyngbya majuscula* occurred at high densities at Town Beach, samples of benthic invertebrates were taken from the three locations indicated above in November 2009 and February 2010.

We followed the methodology used in the Monitoring Roebuck Bay Benthos program (MONROEB, de Goeij *et al.* 2003, de Goeij *et al.* 2008) which has been used in the bay for the last 14 years. Two stations were defined at each site, one 150 m offshore and the second 250 m offshore, perpendicular to the coast. At each station four samples were taken, each one consisting of six 10.3 cm diameter cores, driven 20 cm into the sediment. Therefore each sample represented a sample surface of 0.05 m^2 and each station represented a sampled surface of 0.2 m^2.

The samples were sieved on the shore initially with a 1 mm sieve to remove most of the coarser sediment and then through a 0.5 mm sieve. The samples were labelled and preserved in 70% ethanol and returned to the laboratory for processing. All samples were processed using a stereomicroscope (10 to 22 X magnification), removing all individuals, identifying to family level, and recording abundance of each family.

The information gathered from both stations in each site was pooled together following de Goeij *et al.* 2003, where the authors compared the abundance and diversity of benthic species between both stations in two sampling sites of Roebuck Bay and obtained similar results. Diversity was determined using the Shannon-Wiener diversity index.

Shorebird foraging behaviour and prey

To evaluate any differences in shorebird foraging behaviour between the end of the dry season and mid wet season, when *Lyngbya majuscula* occurred at high densities in Town Beach, observations of Bar-tailed Godwits *Limosa lapponica* chosen randomly and foraging actively were made in November 2009 and February 2010 at Town Beach and One Tree stations. Bar-tailed Godwits are long distance migrant animals and human induced changes in their non-breeding habitats could have detrimental effects at a population level, as has been proven for other long distant migratory shorebirds (Piersma 2006).

Each individual was followed during a three-minute period through a Kowa 25 x 60 telescope ($n^{TB}_{Nov}= 32$; $n^{TB}_{Feb}= 28$; $n^{OT}_{Nov}= 25$; $n^{OT}_{Feb} =30$) three hours before and after low tide. Similar numbers of observations were taken throughout the daylight period (5–19 GMT) before and after low tide to evade the potential mudflat exposure period effect on feeding rates (Rogers and de Goeij 2006). To avoid pseudo-replication (Hulbert 1984), each new bird selected for observation was at least 20–40 m from the previous individual.

During these observations the following variables were recorded: 1) number of pecks (only the tip of the bill entered the substratum), 2) number of probes, 3) number of successful prey captures, 4) prey type (unidentified prey were excluded from diet analysis), 5) depth of probes and 6) depth of captured prey. Note that depths for (5) and (6) were recorded as one of the following three categories: i) only the tip of the bill entered the substratum, ii) less than, or half of the bill entered the substratum and iii) more than half of the bill entered the substratum. The depth of captured prey was then established in relation to the mean bill length of Bar-tailed Godwits in North Western Australia (97.4 mm for both sexes combined; Wilson *et al.* 2007). Consequently, it was considered that when only the tip of the bill entered the substratum, the prey was at a depth between 0 and 24.4 mm, when less than or half of the bill entered the substratum, the prey was at a depth between 24.5 and 48.7 mm and when more than half of the bill entered the substratum, the prey was at a depth between 48.8 and 97.4 mm.

Statistical analysis

Normality and homoscedasticity were tested (Shapiro-Wilk and Levene's test respectively) for each variable. When normality of the data was not achieved a $\log_{10}$ (x+0.1) transformation was applied (Sokal and Rohlf 1995). The differences in abundance of invertebrates, probes per minute and prey captured per minute between months (fixed factor) and among sites (fixed factor) were analysed using a two-way ANOVA test. Differences in prey captured per minute in relation with prey depth were analysed using one-way ANOVA. When analyses showed significant differences, post-hoc tests (parametric Tukey's test) were used to determine amongst which months or sites differences existed.

Values are presented as means ± SE, unless stated otherwise. Statistical significance was set at $P \leq 0.05$. All univariate statistical tests were conducted using Statistica 7.0 (StatSoft. Inc., Tulsa, Oklahoma, USA).

Differences in the composition of the benthic invertebrate assemblage among sites with and without *Lyngbya* were explored using non-metric multidimensional scaling (nMDS) on square root transformed data, using the adjusted Bray-Curtis similarity coefficient (Clarke 1993). The statistical significance of these differences was determined by analysis of similarities (ANOSIM; Clarke 1993). All multivariate analyses were conducted using PRIMER v6 (PRIMER-E Ltd., Lutton, Ivybridge, UK).

Results

Benthic invertebrate diversity and abundance

A total of 1057 and 3389 benthic invertebrates were collected in November 2009 and February 2010 respectively. Of these, 11 main invertebrate taxa were identified. Polychaete worms compromised more than 30% and bivalves more than 20% of the total fauna in November. In February more than 40% of the fauna were sipunculids and almost 24% were gastropods. The total abundance of macrobenthos in November was 1602.5,

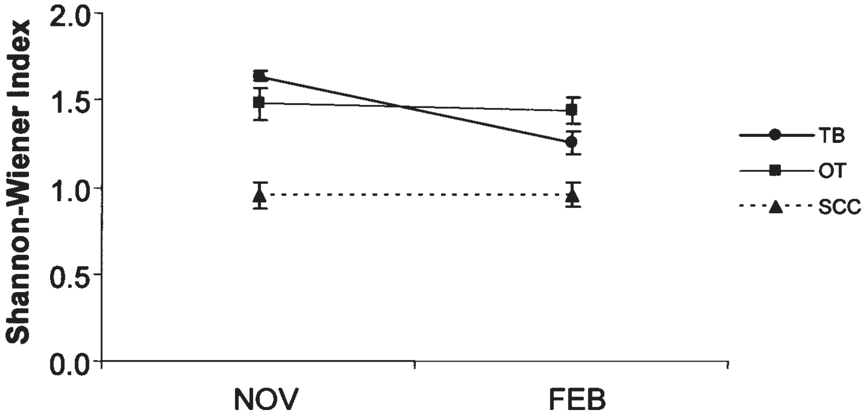


Figure 2. Mean values (±SE) of the Shannon-Wiener diversity index for benthic invertebrates in Town Beach (TB), One Tree (OT) and south of Crab Creek (SCC) in Roebuck Bay, North Western Australia in November 2009 and February 2010.

650.0 and 67.2 individuals per m^2 for Town Beach, One Tree and south of Crab Creek respectively. In February the total abundance was 5117.5, 1437.5 and 88.4 individuals per m^2 for Town Beach, One Tree and south of Crab Creek respectively.

Diversity of invertebrates was significantly different among sites (two-way ANOVA: $F_1 = 5.6$ $P < 0.05$), between months (two-way ANOVA: $F_2 = 35.0$ $P < 0.0001$) and also among sites x months (two-way ANOVA: $F_2 = 4.6$ $P < 0.05$). Diversity of invertebrates was significantly lower at the station south of Crab Creek than in the other two stations in November and February (Fig. 2). There were no changes in invertebrate diversity between November and February in One Tree and south of Crab Creek, but there was a significant decline in diversity in Town Beach from November to February (Fig. 2).

There were significant differences among sites for all the taxa studied (Table 1). Polychaetes, tanaids, ostracods, ophiurids (brittle stars) and isopods were significantly more abundant in Town Beach than in the other two stations (Table 1, Figures 3a, 3b, 3c, 3d, 3e). Bivalve abundance was lower south of Crab Creek in both months (Table 2, Fig. 3f). The abundance of scaphopods was higher in One Tree than in the other two stations (Table 2, Fig. 3g).

There were significant differences in polychaete, brittle star, gastropod and sipunculid abundance between November and February (Table 1). There was a

Table 1

Table 1: Differences in the mean abundance of invertebrates between November 2009 and February 2010 in Town Beach, One Tree and south of Crab Creek in Roebuck Bay, North Western Australia. Asterisks indicate differences between months, among sites or among sites x months (*: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$; n.s.: non-significant).

Taxa	Site		Month		Site x Month	
	F	p	F	p	F	p
Polychaeta	25.97	***	45.91	***	0.69	n.s.
Crabs	5.16	**	0.85	n.s.	0.37	n.s.
Bivalves	29.22	***	0.76	n.s.	0.49	n.s.
Gastropods	9.38	***	3.77	***	3.79	***
Amphipods	9.44	***	0.69	n.s.	24.59	***
Ophiurids	34.54	***	12.65	***	4.03	*
Ostracods	10.29	***	0.40	n.s.	0.67	n.s.
Isopods	8.50	***	1.08	n.s.	1.08	n.s.
Sipunculids	73.07	***	126.69	***	73.07	***
Tanaids	55.93	***	0.26	n.s.	0.26	n.s.
Scaphopods	7.87	**	0.59	n.s.	0.65	n.s.

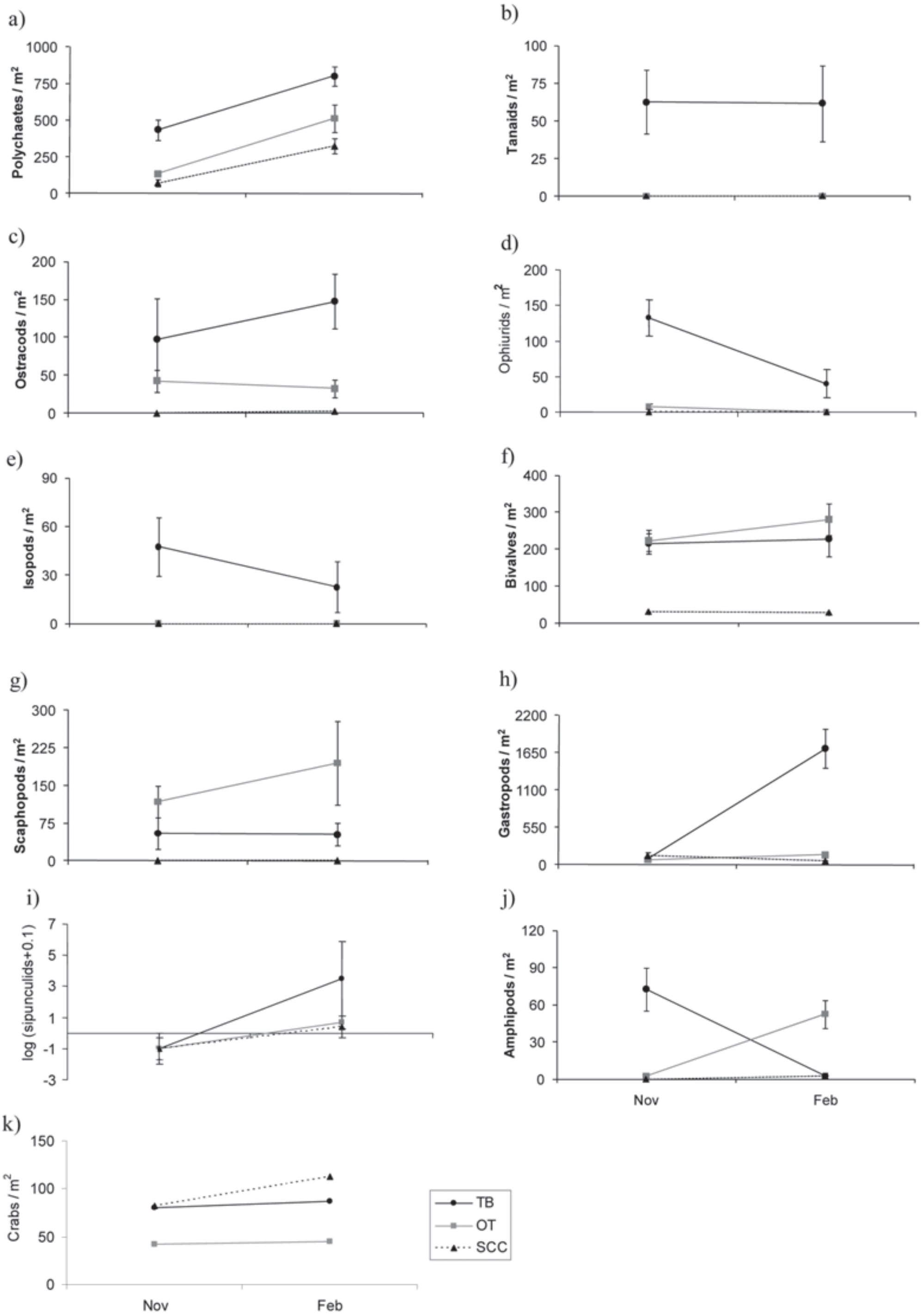
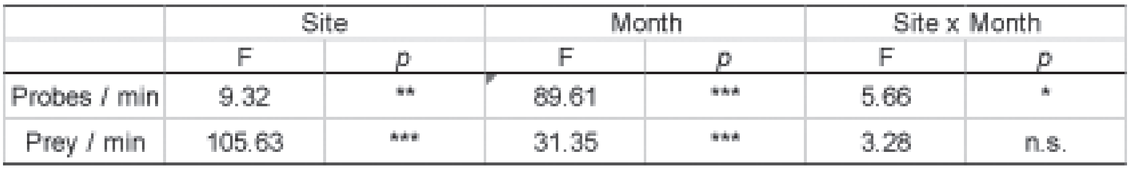


Figure 3. Mean abundance (±SE) of benthic invertebrate taxa grouped as a) polychaetes, b) tanaids, c) ostracods, d) brittle stars, e) isopods, f) bivalves, g) scaphopods, h) gastropods, i) sipunculids (as log (v+0.1)), j) Amphipods and k) crabs in Town Beach (TB), One Tree (OT) and south of Crab Creek (SCC) in Roebuck Bay, North Western Australia, in November 2009 and February 2010.

Table 2

Table 2: Differences in the number of probes per minute and prey captured per minute by Bar-tailed Godwits *Limosa lapponica* in Town Beach and One Tree between November 2009 and February 2010 in Roebuck Bay, North Western Australia. Asterisks indicate differences between months, among sites or among sites x months (*: $p < 0.01$; **: $p < 0.001$; ***: $p < 0.0001$; n.s.: non-significant).



	Site		Month		Site x Month	
	F	*p*	F	*p*	F	*p*
Probes / min	9.32	**	89.61	***	5.66	*
Prey / min	105.63	***	31.35	***	3.28	n.s.

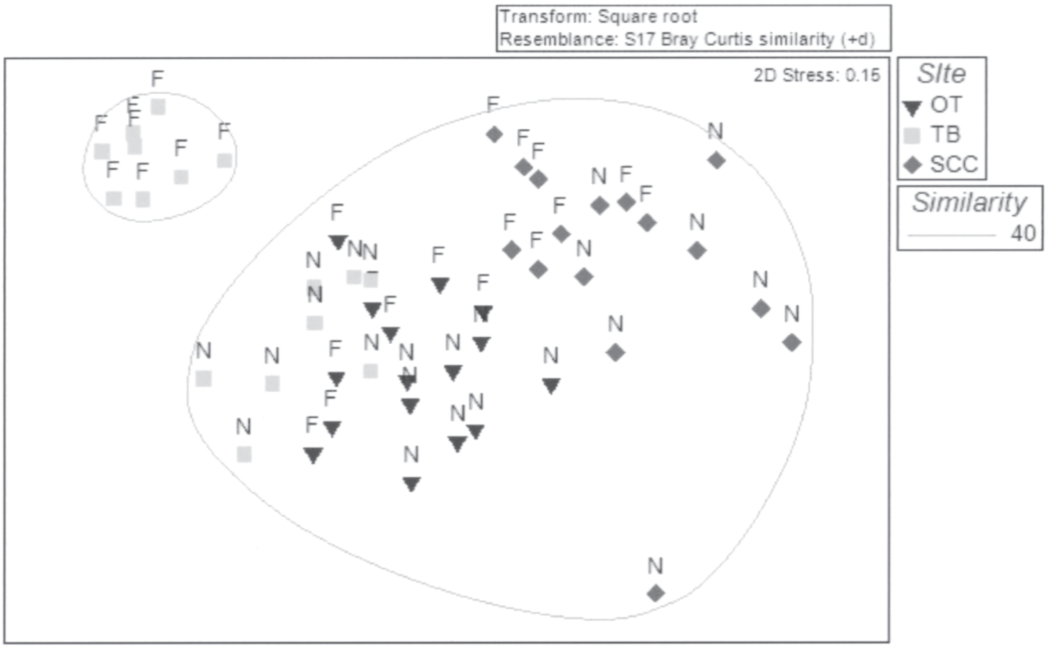


Figure 4. n-MDS ordinations using the adjusted Bray-Curtis similarity measure on square root transformed abundance data from core samples collected in Town Beach (TB), One Tree (OT) and south of Crab Creek (SCC) in Roebuck Bay, North Western Australia, in November 2009 (N) and February 2010 (F).

significant increase in the abundance of polychaetes in all the stations from November to February (Fig. 3a). There was also a significant increase in the abundance of gastropods and sipunculids at Town Beach between November and February; the abundance of both taxa was significantly higher in Town Beach in February than it was at the other stations at any time (Table 1, Figures 3h, 3i). In contrast there was a decrease in amphipods between November and February in Town Beach; the abundance of amphipods in Town Beach in November was significantly higher than in all other samples except in One Tree in February, whereas the abundance of amphipods was also high (Table 1, Fig. 3j). There was a decrease in brittle stars between November and February in Town Beach. The abundance of brittle stars in Town Beach in November was significantly higher than in all other samples, whereas in February the abundance of brittle stars in Town Beach was similar to the abundance of brittle stars in One Tree in November (Table 1, Fig. 3d). There was no significant change in abundance of crabs at any site between both months (Table 1, Fig. 3k).

Ordination analysis of the benthic invertebrate abundance data showed that Town Beach samples from February grouped together in a distinct and coherent group separate from the rest of the samples of Town Beach sampled in November and the samples of One Tree and south of Crab Creek sampled in November and February (Fig. 4). The ANOSIM test also showed that there was a significant difference among the sites with *Lyngbya* in February (Town Beach) and the sites without *Lyngbya* in February and November (Global $R = 0.64$, $P = 0.1$).

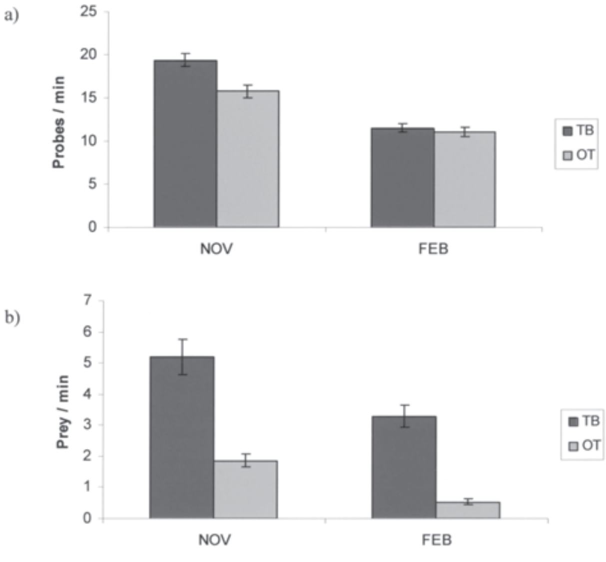


Figure 5. Mean (±SE) number of probes and prey capture per minute of Bar-tailed Godwit feeding on benthic invertebrates in Town Beach (TB) and One Tree (OT) in Roebuck Bay, North Western Australia, in November 2009 and February 2010.

Shorebird foraging behaviour and prey

There were significant differences in the number of probes and prey captured per minute between Town Beach and One Tree and between November and February. However the number of probes per minute in Town Beach and One Tree were similar in February (Table 2 and Fig. 5a and 5b).

There were also significant differences in the number of prey captured per minute depending on the depth of captured prey in both sites in both months (Fig. 6). Godwits captured more prey when they fed on deeper-

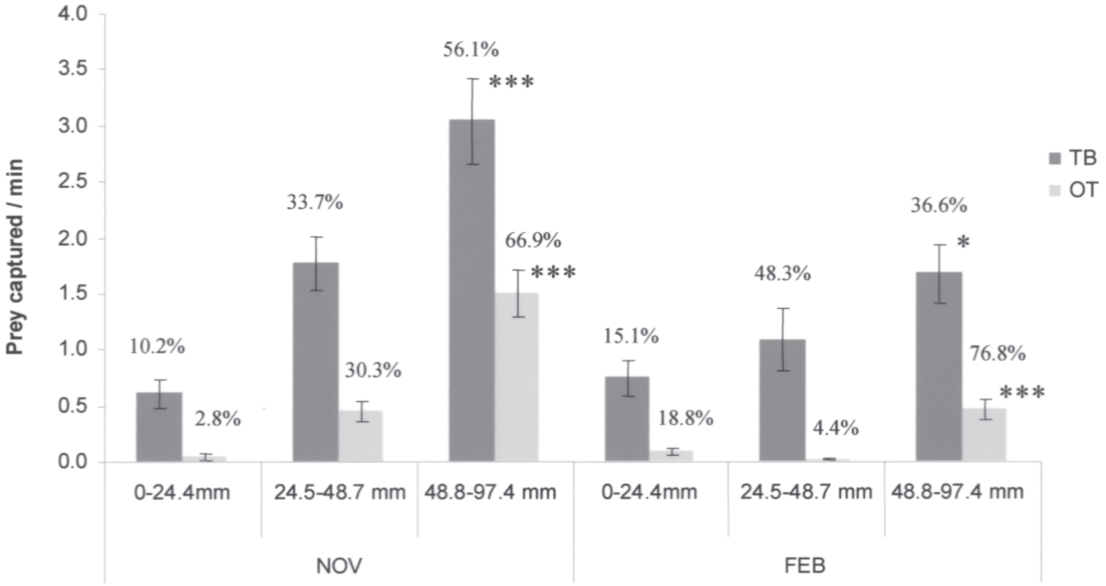


Figure 6. Mean number (±SE) of prey captured per minute by Bar-tailed Godwit depending on the depth of captured prey in Town Beach (TB) and One Tree (OT) in Roebuck Bay, North Western Australia, in November 2009 and February 2010. Asterisks indicate significant differences in the number of prey captured among the different depths in each site for each month (One-way ANOVA *: $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$). The percentage number on top of each bar indicates the percentage of prey captured at each depth in each site for each month.

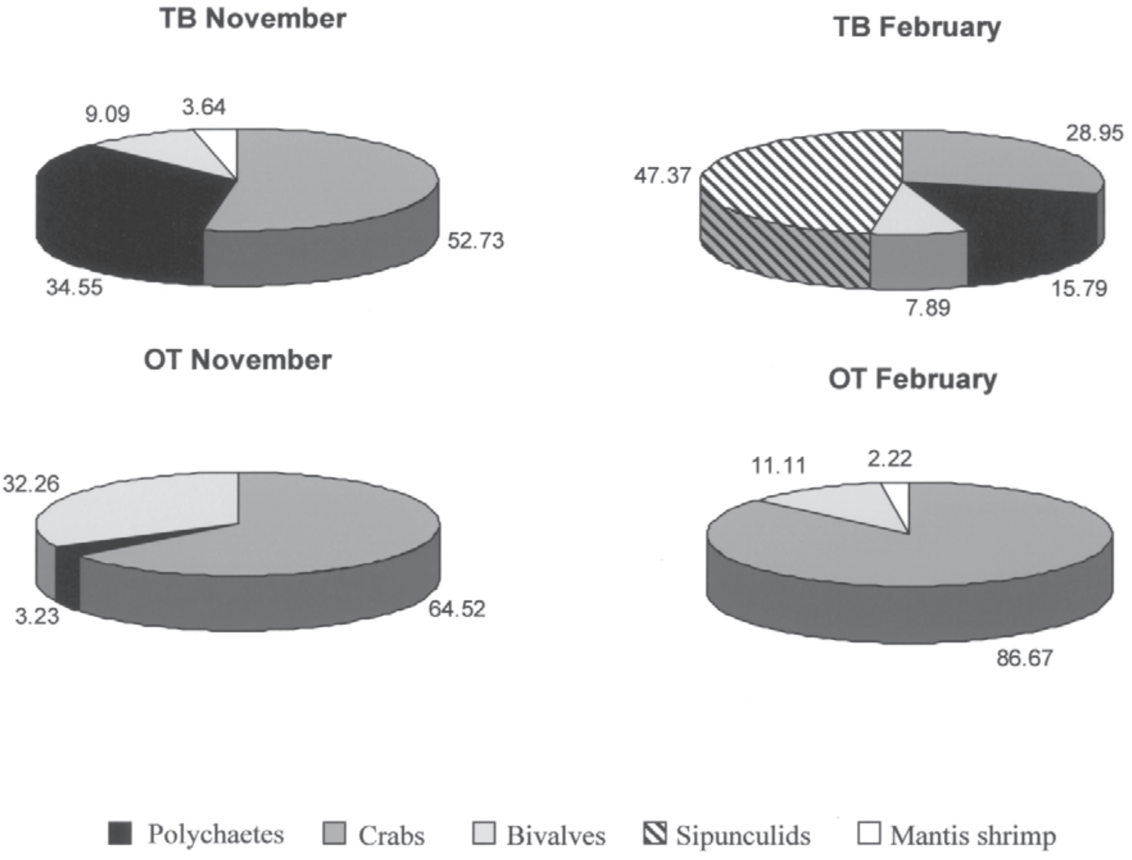


Figure 7. Percentages of the different prey captured by Bar-tailed Godwit feeding in Town Beach and in One Tree in Roebuck Bay, North Western Australia in November 2009 and February 2010. The percentages have been obtained from direct observations of Bar-tailed Godwits feeding actively three hours before and after low tide.

buried prey in both months in both stations (Fig. 6). However, although most of the prey (>55% of captured prey) were captured in the 48.8 to 97.4 mm depth category in both sites in November and in One Tree in February, in Town Beach in February 48% of the prey were captured in the 24.5 to 48.7 mm depth category (Fig. 6).

In November, the most common prey for Bar-tailed Godwits were crabs at both Town Beach and One Tree. The second most common prey were polychaetes and bivalves in Town Beach and One Tree respectively (see Fig. 7). In February, crabs were also the most common prey for godwits in One Tree, whereas in Town Beach sipunculids were most commonly taken, followed by crabs (see Fig. 7).

Discussion

Nutrient enrichment can significantly alter biodiversity, producing for example shifts in assemblages of primary producers and favouring cyanobacterium blooms which are associated with episodes of anoxia and hypoxia. Town Beach was the only site where *Lyngbya* was present in February, and it was the only site where the diversity of benthic invertebrates showed a significant decrease between November and February. This appears to be linked with the dramatic increase in abundance of sipunculids (from 0 to 3157.5 sipunculids per m^2) and gastropods (from 82.5 to 1715 gastropods per m^2) in Town Beach, both taxa considered as being tolerant of anoxic conditions (Langenbuch and Pörtner 2004; Vaquer-Sunyer and Duarte 2008). Also, while the community assemblage of Town Beach did not appear different to the other two sites in November, it was significantly different in February. Overall, our preliminary results suggest that *Lyngbya* blooms are affecting the benthic invertebrate community on some areas of Roebuck Bay. These results are in line with what was found in Moreton Bay (Queensland), where *Lyngbya* blooms also affected significantly the abundance of several benthic invertebrates (García and Johnstone 2006). Nevertheless changes in the distribution and abundance of benthic organisms could also be attributed to natural seasonal patterns. For example, the changes observed in the abundance of polychaetes appear to be more related to expected seasonal variation in a tropical tidal flat, where higher densities occur in the wet season (Metcalfe and Glasby 2008).

Therefore, a more detailed benthic sampling program with more sampling events, including environmental variables such as nutrient concentrations and sediment grain size as well as better taxonomic resolution is required in order to provide a more precise conclusion.

Apart from observed changes in polychaetes, there were no significant changes in the abundance of the other two main Bar-tailed Godwit prey items. Although there was no apparent *Lyngbya* induced change in prey of godwits, there was a change in godwit foraging behaviour in Town Beach from November to February. In November in both sites and in February in One Tree, Bar-tailed Godwits captured most of their prey at the maximum depth range and fed mostly on crabs. Yet in February in Town Beach when *Lyngbya* was present almost 50% of Bar-tailed Godwit prey was captured at a depth between 24.5 and 48.7 mm, and sipunculids were the main prey item. Sentinel crabs, the most abundant crab in the tidal flats of Roebuck Bay (de Goeij *et al.* 2003; de Goeij *et al.* 2008; Piersma *et al.* 2006) probably hide in their borrows at a higher depth than sipunculids, found in Town Beach only in the first centimetres of the sediment (SME personal observations). As in other situations, this change in behaviour suggests that godwits used an opportunistic foraging strategy (Davis and Smith 2001; Skagen 2006) to exploit the available, high density and low mobile sipunculids. However the implications of this shift in prey on this long distance migratory bird with high energetic demands need to be studied in depth to fully understand the potential impacts of *Lyngbya* presence on shorebirds. For example, if sipunculids have a lower energetic value per individual than more profitable prey items (*i.e.* crabs and polychaetes) this may affect the ability of birds to accumulate energy reserves. Also, the fact that godwits capture more prey per minute in Town Beach than in One Tree does not mean that the actual energy intakes are higher at Town Beach. Shorebirds can achieve higher intake rates in sites where they capture fewer but more profitable prey (Rogers and de Goeij 2006).

Finally based on these initial results a more in depth study is currently underway, involving more sampling occasions, using stable isotopes analysis, mapping *Lyngbya* extension and assessing nutrient concentrations in sediments to better elucidate the effects of *Lyngbya* blooms on benthic fauna, shorebird foodwebs and possible relationships with nutrient sources.

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