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#### Licenses

The benthic invertebrate samples and the shorebird's blood samples were taken under the licenses to take fauna for scientific purposes number SF007116 and SF007246 of the Department of Environment and Conservation of Western Australia. The shorebird observational study was approved by the Animal Ethics Committee of the University of Western Australia (File ref.:F18979). The shorebird blood extraction was approved by the Animal Ethic Committee of the University of Western Australia (File ref.: RA/3/100/907).

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#### **Cover pictures:**

- Lyngbya majuscula at Roebuck Bay. Tom de Silva
- Roebuck Bay. Sora M. Estrella
- Flock of shorebirds roosting in Roebuck Bay. Jose A Masero
- Blue crab, fan worm and Great Sand Plover. Tom de Silva

#### Disclaimer

Prepared for the NRM Office by:

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### **Summary**

From 2005, blooms of *Lyngbya majuscula* have been occurring in the intertidal zone of Roebuck Bay. A significant concern is the effect that *Lyngbya* blooms and nutrient enrichment events may have in Roebuck Bay ecosystem, particularly given Roebuck Bay was designated as a Wetland of International Importance in 1990 under the Ramsar Convention (1971), currently ranking in the top eight shorebird sites in the world. In recognition of its ecological richness, Roebuck Bay was proposed as a Marine Park by the State government in 2010. It is, therefore, a highly significant bird habitat worthy of preservation at a national and international level. Comprehensive and effective management of the Bay is fundamental to a successful future Marine Park and ecosystem.

The "Effects of nutrient enrichment and toxic Lyngbya blooms on benthic invertebrates-and migratory shorebird communities of Roebuck Bay Ramsar site" has been a three-year research project to improve knowledge and provide a scientific framework for nutrient and *Lyngbya* management. The project, funded by the NRM Office, DEC Kimberley, Port of Broome and NRM Rangelands, aimed to gain an understanding of the impacts that *Lyngbya majuscula* may have on the ecology of the Bay, and the mechanisms that drive *Lyngbya* blooms.

This report presents the results and conclusions of the *Lyngby*a and nutrients project in Roebuck Bay.

The high levels of nutrients in water and sediment found, above water quality guidelines together with the opportunistic blooms of the cyanobacteria *Lyngbya majuscula* are indicative of nutrient enrichment and pose a potential problem of eutrophication. The nutrient enriched sediments of the Bay were related with a food web enriched in nitrogen.

Blooms of *Lyngbya majuscula* had significantly affected and modified the benthic invertebrate community of Roebuck Bay which had a cascade effect on the foraging behaviour of at least one species of shorebird, the Bar-tailed Godwit (*Limosa lapponica*), whose diet was modified when exposed to high density *Lyngbya* blooms.

Blooms of *Lyngbya* in Roebuck Bay are dependent on concentrated heavy rains in December, extended periods of sunny days within the same month, warm temperatures in January and sediments rich in ammonium and phosphorous.

Since it is not possible to control the bloom once it has developed, the appropriate approach is to implement management actions to prevent bloom formation. Data from this and other studies indicate that nutrient levels in the Bay are elevated above water quality guidelines. Eutrophication is an acknowledged driving force for algal blooms, and therefore the main recommendation is to avoid eutrophication of the system, and thereby make nutrients limiting; this clearly means to reduce the input of nutrients into Roebuck Bay. However, the lack of knowledge about nutrient sources and the hydrodynamics of Roebuck Bay make this task difficult. Further work is required to identify the source(s) of nutrients entering the Bay, and then implement management actions to reduce nutrient loads to the system, and also to understand the hydrodynamics of the Bay, especially circulation patterns, tidal currents and extent of flushing.



Lyngbya majuscula growing on seagrass beds at Roebuck Bay. (Photo: S.M. Estrella)

# **INTRODUCTION**

#### INTRODUCTION

#### **Background**

A major challenge in ecology and conservation research is to improve our understanding of the diversity and function of ecosystems to develop proper protection, monitoring and management programs that assure their existence for future generations. Because coastal ecosystems exist in the boundary between the ocean and land, they represent an amalgamation of different habitats (e.g. reefs, seagrass beds, salt-marshes and mangroves). As a result, coastal ecosystems are heterogeneous, often being characterised by high levels of production (Gattuso et al. 1998, Borjes et al. 2006) and a high diversity of ecological processes (Constanza et al. 1993). The high primary production in these zones is associated with a varied diversity of primary producers because shallow waters allow the development of different types of macrophytes (Dubois et al. 2012). However, coastal habitats are also subjected to high anthropogenic pressure, specillay in Australia where the majority of the population lives along the coast. As a result coastal ecosystems represent the most endangered ecosystems in the world (Duarte 2007). More than one third of the human population of the world 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). Therefore, understanding the effects that human activities have on these ecosystems is of primary importance. Pressures such as overharvesting of marine organisms, land reclamation and more recently, nutrient loading and climate change are pervasively changing, degrading or destroying coastal wetland ecosystems throughout the world (Agardy et al. 2005).

Anthropogenic nutrient enrichment of wetlands has become a prime issue for both scientists and managers. It is well establish that nutrient enrichment can significantly alter biodiversity, producing for example shifts in the assemblages of primary producers and favouring

phytoplankton, cyanobacteria or macroalgal blooms which may be the cause of episodes of anoxia and hypoxia. Changes in the assemblages of primary producers consequently affect, and often result in the loss of the primary consumers that depend on them (Tewfik et al. 2005). However, the consequences of increasing eutrophication for higher trophic levels are not always apparent (Philippart et al. 2007).

#### **Roebuck Bay**

Roebuck Bay is one of the main wintering and refuelling stop-over areas for migratory shorebirds of the East Asia-Australasia fly way. Roebuck bay is located south of the town of Broome, in North-western Australia, which is the main site for migratory shorebirds on the Australian continent. Roebuck Bay is characterised by a high tidal range (10 m on spring tides), and extensive mudflats. The importance of Roebuck Bay as a shorebird site appears to relate to the incredibly high diversity and biomass of benthic invertebrates in the intertidal flats, which places this tropical intertidal area among the richest mudflats in the world (Piersma et al. 1998). Worldwide there are around twelve sites where large mudflats rich in shorebirds are found at low tide and, only two are located in the tropical region, Roebuck Bay being one of them (Rogers 2003). The number of shorebirds using Roebuck Bay may exceed 120 000 in the non-breeding season (C. Hassell, personal communication) and it is the most important shorebird site in Australia due to the number of species it supports in internationally significant numbers (Rogers et al. 2003). Roebuck Bay was designated as a Wetland of International Importance in 1990 under the Ramsar Convention (1971), and it currently ranks in the top eight shorebird sites in the world (Rogers et al. 2003). Resembling its ecological richness, Roebuck Bay was proposed as a Marine Park by the State government

in 2010. It is, therefore, a highly significant bird habitat worthy of preservation at a national and international level.

However, Roebuck Bay is adjacent to the tourist town of Broome (14,500 inhabitants), and recent studies in Roebuck Bay indicate a developing issue with respect to nutrient contamination. A study of regional groundwater has shown elevated nutrient levels in groundwater originating from the vicinity 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 (Storey unpub. data), blooms of cyanobacteria (blue-green algae) *Lyngbya majuscula* first appeared in 2005 and have occurred to varying degree each year since then, and preliminary assessment of the nutrient loads in sediments adjacent to Town Beach indicating elevated levels of P and N (RBWG 2008), indicative of nutrient enrichment.

#### **Nutrient enrichment**

Nutrient entrance in coastal habitats is a natural process that occurs as a result of runoffs from non-transform natural hinterlands and inputs from ocean upwelling. However, population growth and related nutrient sources such as wastewater treatment plants, and urban and agricultural runoff have increased nutrient inputs to the many aquatic ecosystems, to the point that eutrophication is now one of the greatest threats to coastal ecosystem health (EC and WHO 2002, NRC 2000). Excessive nutrients can lead to serious impacts including blooms of opportunistic primary producers, loss of submerged aquatic vegetation, low dissolved oxygen concentrations and changes to the invertebrate and vertebrate communities these systems support (Hauxwell et al. 2000; Bowen and Valiela 2001; Bricker et al. 2008; Tewfic et el. 2007).

Increased nutrients loads to shallow waters enhance the proliferation of faster growing phytoplankton, macroalgae or cyanobacteria, which then compete with seagrass for light and space. Excessive growth of nuisance plants may smothering and kill seagrass beds. Most of these effects have been studied experimentally or in situ when macroalgal blooms have developed on seagrass beds (Hauxwell et al. 2000, Leoni et al. 2007). The prime mechanism for the loos of seagrass beds is light attenuation due to the macroalgal covering the seagrass (Hauxwell et al. 2000, Brun et al. 2003, Thomsen et al. 2011). Other secondary effects are an increase in the concentration of ammonium derived from the breakdown of senescent macroalgal, which can be toxic for seagrass (van Katwijk et al. 1997), and the anoxic and reducing conditions created in the sediments by the decomposition of the dead macroalgal, is also known to affect seagrass (Duarte 2002). The loss of the seagrass beds and their replacement by other fast growing primary producers leads to other changes that propagate up through the coastal food webs and have negative effects at the community and ecosystemlevel (Fox et al. 2010, McClelland and Valiela 1997). Decreased in abundance and diversity of fishes and invertebrates is often observed when blooms of macroalga affect seagrass beds (Valiela et al. 1992, Ahern et al. 1995, Raffaelli et al. 1998).

#### Lyngbya majuscula

The toxic cyanobacteria *Lyngbya majuscula* (blue-green algae), from the order Oscillatoriacea, is a natural inhabitant of sub-tropical and tropical coastal and estuarine areas of the world. It is a filamentous, non-heterocystous nitrogen-fixing marine cyanobacterium (although evidence of a non nitrogen-fixing strain of *L. majuscula* L3 has been reported, see Jones et al. 2011). In the presence of excess nutrients and good weather (i.e. clear skies and warm water temperatrures) *L. majuscula* will rapidly grow at an exponential rate and is

commonly referred to as a "bloom event" (Johnstone et al. 2010). Bloom can lead to major ecosystem changes and have been known to affect seagrass growth (Paerl et al. 2009), meiofauna community (García and Jhonstone 2006), fish (Pittmann and Pittman 2005), oxygen levels in the water column (García and Jhonstone 2006) and aesthetics and social/amenity use of the affected area (Watkinson et al. 2005). In recent years blooms of *L. majuscula* are occurring more often, with more intensity and greater spatial coverage in tropical and subtropical marine ecosystems (Paerl et al. 2009). This increase in the occurrence of cyanobacteria blooms has been linked to human induced eutrophication (principally from land-based nutrient loads) of the aquatic systems, and more recently with climate change (Paerl et al. 2011). Rising temperatures will favour cyanobacteria blooms since cyanobacteria normally exhibit optimal growth rates at high temperatures (Paerl and Husiman 2009). In fact, recent models for an enclosed sea have revealed that with climate change and increased temperatures, the number of days favouring cyanobacteria blooms will likely increase, and therefore anoxic events may become more frequent and last longer (Neumann et al. 2012).

Severe blooms of *Lyngbya majuscula* will limit ambient light reaching the seagrass and other primary producers, with their consequent smothering (Watkinson et al. 2005). Changes in the primary producers can then affect the primary consumers that depend on them (Tewfik et al. 2005). Also, *Lyngbya* blooms often result in oxygen depletion (Denison and Abal 1999), which may have significant consequences at ecosystem level, affecting for example fisheries (Pittman and Pittman 2005) and habitat quality. Despite of these recognise direct and indirect effects of *Lyngbya* blooms, little is known about its environmental consequences and only few studies have evaluated the effects of *Lyngbya majuscula* and other marine cyanobacteria blooms on aquatic fauna (Butler et al. 1995, Pittman and Pittman 2005, García and Johnstone 2006, Arthur et al. 2006, Arthur et al. 2008a, b, Gilby et al. 2011) or food webs.

Several cyanobacterias, including the genus *Lyngbya*, are able to produce toxins. These toxins may have detrimental effects on the marine ecosystems and are also known to be a potential human health risk. In Moreton Bay, southeast Queensland for example, *Lyngbya* blooms were related with skin reactions, headaches and breathing problems (Osborne et al. 2001). At ecosystem level, some studies have link *Lyngbya* blooms and its toxins with Green Turtle skin tumours and changes in the blood biochemist of turtles (Arthur et al. 2008a, b).

Lyngbya majuscula occurrence has been recorded in Australia at a number of locations, especially along the Queensland coast (Albert et al. 2005). In Moreton Bay the increasing frequency, severity and extension of the blue-green algae blooms set off the Lygnbya Research and Management Program. One of the main findings of the project was the identification of the main triggers for Lyngbya blooms in Moreton Bay, being the available dissolve nutrient pool, specially dissolve P and Fe, levels of nutrients in sediments, dissolve organic carbon, light and temperature (Johnson et al 2010).

#### Scope of the study

The present study was conceived as an integrated and multidisciplinary investigation of the effects that human activities, in this case, nutrient enrichment and the resulting *Lyngbya* blooms, have on the food web of shorebirds of the Roebuck Bay ecosystem.

Following concerns that different researchers as well as community members and organizations had on the ecological health of Roebuck Bay, together with the limited data that indicated there was nutrient enrichment of the Bay, a list of issues that required scientific study was developed and became the basis of the present study objectives.

The study posed five different tasks:

-Preliminary study (2009-2010): Funded by Department of Environment and Conservation (DEC) Kimberley and Port of Broome Authority, with logistic support of DEC Broome, the Broome Bird Observatory (BBO), Global Flyway Network, the Australasian Wader Studies Group and approximately 30 volunteers.

-Main field and laboratory work (2010-2012): Funded by NRM Office, DEC Kimberley, NRM Rangelands and Port of Broome Authority, with logistic support of DEC Broome, BBO, Global Flyway Network, AWSG and approximately 60 volunteers.

-Creation of data base and data analysis. Progress reporting (2009-2013).

-Community and media information: Community Program, Bay Day, Roebuck Bay Working Group (RBWG) Keep Our Bay Clean, Newsletters, final report, media interviews (newspapers and ABC Radio). This activity will continue by the main researcher of the project through the RBWG after the project finishes.

-Roebuck Bay Lyngbya Monitoring Program.

-Final report.

#### Objectives of the study

This project aimed to understand the impacts that *Lyngbya majuscula* may have on the ecology of the Bay, and the mechanisms that drive *Lyngbya* blooms.

This general aim was reached through the following partial objectives:

1. Monitor the frequency, duration, intensity (biomass) and extension of the algal blooms and endeavour to identify chemical, physical or biological triggers.

- 2. Evaluate the potential effects of nutrient enrichment on benthic invertebrates and shorebirds in Roebuck Bay.
- 3. Assess the possible impact of *Lyngbya majuscula* on benthic biota and shorebird feeding ecology.
- 4. Generate recommendations for a management plan for *Lyngbya majuscula* blooms and nutrients inputs in the Bay.

To carry out the above objectives, samples of benthic fauna within and outside areas of *Lyngbya* were collected to assess effects of blooms on benthos. Nutrient levels in sediments/water were asses to trace enrichment and relate them to *Lyngbya* blooms. Observations of feeding behaviour of shorebirds were obtained to determine how behaviour was affected by blooms. Finally a collection of primary producers and primary and higher consumers for stable isotope analysis, including blood samples from 260 birds was carried out to track nutrient enrichment through the food web.

#### Associated studies, community actions active in Roebuck Bay and collaborations

Several studies and community actions are active in Roebuck Bay that have led to current or future cooperation or that could receive some input from the information provided by this study.

The Roebuck Bay Working Group (RWG) is the major community and stakeholder organization for Roebuck Bay, with the main objective being to protect the Bay's environment. It has become one of the main partners in the development of the present study and has served as an active and effective way of communication with the community. An ongoing collaboration has produce the successful project "Keep Our Bay Clean", with the

main purpose of reducing pollutants from entering the Bay (Funded by NRM Rangelands). With the greater RBWG project is the program "Prevent *Lyngbya* blooms".

The State Department of Environment and Conservation (DEC) is a co-founder and collaborator of the study. It has provided logistic and human resources along the study. Yawuru Rangers, an indigenous ranger group, have assisted with the project with benthic sampling sediment and water nutrient sampling and *Lyngbya* mapping. They have also received formation on the field procedures and methodology. The Roebuck Bay *Lyngbya* Monitoring Program provided by this study will be the main tool for DEC Broome in *Lyngbya* monitoring. Also the Management Recommendations will serve as a future guide for management of *Lyngbya* blooms.

The NRM Rangelands is a co-founder and collaborator of the study. It has provided resources along the study to the RBWG to carry out the project "Keep Our Bay Clean". Also, it has provided important information about potential nutrient loading points to the Bay.

The Seagrass Monitoring Program coordinated by Environs Kimberley, will this year carry the *Lyngbya* Monitoring Program using the information provided in the Roebuck Bay *Lyngbya* Monitoring Program.

The study by PhD researcher, Gayan Lakendra Gunaratne under the supervision of Assoc. Prof. Ryan Vogwill, Assoc. Prof. Matt Hipsey and Assoc. Prof. Ryan Lowe "The effects of altered hydrological regimes on water quality and nutrient delivery to a sub-tropical coastal transitional wetland" would cover different aspects of the hydrological processes associated with nutrient discharge to Roebuck Bay and the hydrodynamics of nutrient flushing of a coastal eco-system. It has been schedule future collaborations with data and information interchange between both projects. Also the Honours project of Thomas de Silva under the

supervision of Dr. Mike Van Keulen, Dr. Navid Moheimani and Dr. Sora M. Estrella would develop further biological implications of *Lyngbya* blooms not cover by the present study.



From left to right and top to bottom: blood extraction from a Great Knot, DEC personnel and volunteers after macrobenthos sampling at One Tree, wet season in Roebuck Bay, shorebirds observations, DEC hovercraft, volunteers and Yawurru Rangers after macrobenthos sampling at One Tree (Photos: Tom de Silva and S.M. Estrella)

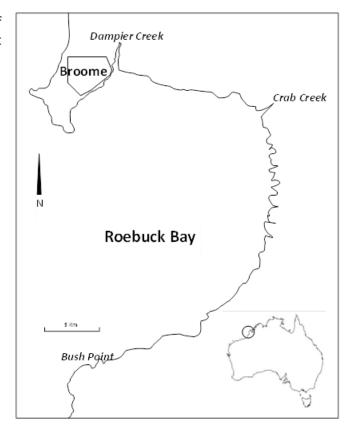
# **METHODS**

#### **METHODS**

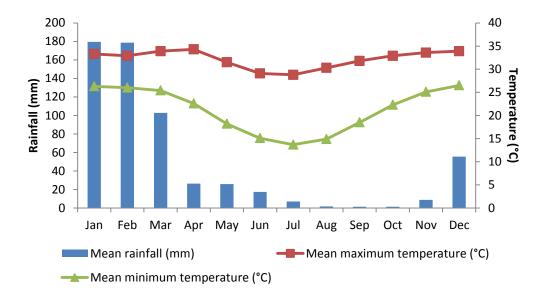
#### Study site

Roebuck Bay (18"011S, 122"24'E) is situated on the North West coast of the Kimberley Region of Western Australia (Figure 1).

**Figure 1.** Map showing the location of Roebuck Bay on the west Kimberley coast of Western Australia.



The area is in the dry tropics of northern Australia, with a monsoonal climate characterised by a dry, warm season from May to October and a wet, hot season from November to March, with transitional periods in between (Figure 2). Most rainfall is derived from northern monsoonal troughs, with occasional high rainfall from tropical cyclones.



**Figure 2.** Mean monthly rainfall and mean maximum and minimum temperature recorded from Broome airport (station # 003003; 1939-2012). Data from the Australian Bureau of Meteorology.

Roebuck Bay is a low energy-tide dominated embayment. It presents a macrotidal regime, with spring tides ranging >10 m, which exposes more than 45% of the tidal flats of Bay area (aprox. 150 Km<sup>2</sup>), but approximately 10% of the Bay at neap tides (Pepping et al. 1990). The Bay presents one of the greatest tidal regimes in world, with only Kind Sound in NW Australia and Bay of Fundy (Canada) having a greater range. However, the tidal current velocities are low due to the geomorphology of the Bay (Pepping et al. 1990).

Through natural process, Roebuck Bay is a nutrient-limited ecosystem. The Leeuwin Current brings low nutrients oceanic waters to the Bay from waters around the Indonesian Archipelago. Also runoff from hinterland is limited, only occurring via sheet flow in the wet season, and terrestrial soils are also poor in nutrients (Pipping et al. 1990). This, together with the turbidity of the water due to suspended fine sediment, limits the primary production of the area. As a result chlorophyll-a concentrations in the surface waters of the Bay are low  $(0.7\pm0.4~\mu g/L)$ , Rose et al. 1990), indicating low phytoplankton productivity, and the seagrass meadows are sparse across much of the Bay, only consisting of small-sized plants (Pepping et

al. 1999). However, there are well developed seagrass meadows in the north west section of the Bay, where turbidity is probably lower due to sediment type, which include the species *Halophila ovalis* and *Halodule uninervis* (Walker and Prince 1987).

The north west part of the Bay is characterised by a relatively narrow intertidal area (1.5 km aprox. in spring tides) consisting of sand flats and seagrass meadows. The shoreline is lined by a narrow fringe of mangroves (mostly *Avicennia marina*), interspersed by sandy beaches and low cliffs. The northwest part of the Bay is separated from the northern beaches by Dampier Creek, a tidal channel supporting a well-developed mangrove system. The northern coast of the Bay is composed by sandy or pebble beaches with Pindan cliffs 15 m high. The intertidal zone of this part of the Bay consists on fine sandy sediments and narrow intertidal flats up to Fall Point (Figure 1). There is a reduce presence of mangroves in this section of the Bay because erosion is the main morphological process in this area (Pipping et al. 1990). From Fall Point to the north east corner (One Tree, see figure 3) and to the south, the intertidal area widens up to 13 km and sediments are soft muddy silt sediments, with some isolate sandbanks to the south. There is a wide and well develop mangrove fringe intersected by numerous tidal creeks up to as far as Bush Point to the southwest. Supratidal flats occur behind the mangrove area. These flats become inundated during wet season king tides, tidal surges from cyclones, and following extensive rainfall.

#### Sampling sites

Two main sampling locations were selected in the intertidal area to characterize: (1) the area potentially directly affected by rum-off from Broome (Town Beach, TB) and (2) an area adjacent to an extensive area of mangroves, which due to its remoteness would act as a control site (One Tree, OT) (Figure 3).

At each site two stations were defined, one 150 m offshore (Site A) and the second 250 m from high tide mark (Site B), perpendicular to the shore. At Town Beach, the 150 m station (TBA) was characterised by a bare sand flat without seagrass (Plate 1A). The second 250 m station (TBB) was characterised by an extensive meadow of seagrass (Plate 1B). This difference in seagrass presence is probably related with the different exposure time at low tide, with TBA being exposed for longer periods and more frequently than TBB depending on the tidal amplitude.

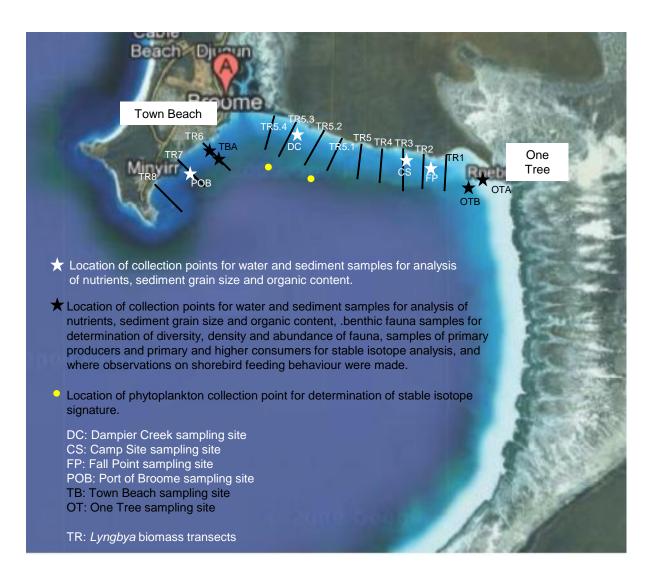


Figure 3. Sampling sites of the study in Roebuck Bay, NW Australia.

At One Tree both stations (OTA and OTB) were characterised by extensive soft mudflats with no macroalgae or seagrass beds present (Plate 2).





**Plate 1**. A) Town Beach station A, 150 m offshore (February 2010). Characterised by a sand flat without seagrass meadows or macroalgae. The black spots on the sand are *Lyngbya majuscula*. B) Town Beach station B, 250 m off shore (November 2009), characterised by an extensive seagrass meadow.



**Plate 2.** Mudflats at One Tree sampling site. There are no differences between the two sampling stations, A (150m offshore) and B (250 m off shore). The black spots in the picture are hundreds of shorebirds feeding (November 2009).

#### Mapping coverage and biomass of Lyngbya majuscula

Temporal changes in the spatial coverage and biomass of *Lyngbya majuscula* was mapped using two different methods, mapping the border of the bloom and collecting quadrat samples along using transects.

In the peak of the bloom that occurred in February each year of the study period, the border of the bloom was mapped by GPS from a hovercraft (DEC hovercraft in 2010, Broome Hovercraft (Holdage Pty. Ltd.) in 2011). In 2012 the limits of the bloom was mapped by foot in several consecutive days.

To quantify biomass of *Lyngbya* in the affected area, 13 transects were set up between the Port of Broome and One Tree, covering the spatial occurrence of the blooms. The distance

between transects was approximately 1 to 1.5 km. Each transect ran perpendicular to the shoreline for 1 km across the intertidal zone and was positioned by GPS (see Figure 3). Every 100 meters, three replicate samples of *Lyngbya* were collected using a quadrat (25cm x 25cm) (Plate 3). At each station the quadrat was randomly positioned within a 5 m radius of the station. All *Lyngbya* within the quadrat was removed and placed in a labelled bag. Surveys were only conducted when there was *Lyngbya* growth evident. In the wet season of 2010-2011 transects were sampled every two months and in the wet season 2011-2012 every month from December to April, providing estimates of *Lyngbya* coverage and biomass in December 2010, February, April and December 2011, January, February, March and April 2012.

**Plate 3**. Quadrat with *Lyngbya majuscula* on a sand flat of Roebuck Bay.



In February 2010, during the pilot study, qualitative samples of *Lyngbya majuscula* were taken in Town Beach and midway between Dampier Creek and One Tree.

The samples were stored in labelled bags and frozen for subsequent analysis. In the laboratory, *Lyngbya* was cleaned of all foreign material, dried for 24 h at 60°C and weighted Dry Mass (DM). The dried sample was then combusted at 500 °C for 2 h in a muffle furnace. Ash Free Dry Mass (AFDM, being a measure of the organic content of the sample) was determined as the difference between the DM and the remaining ash.

## Water and sediment nutrients' concentration

To identify spatial and temporal variations nutrient status, samples of water and sediment were collected at low tide from different sampling sites across the study area (see Figure 3). In the wet season of 2010-2011 samples for nutrient analysis were sampled every two months and in the in the wet season of 2011-2012 sampling was conducted every month from December to April inclusive.

Three replicate samples were collected from each site except sites where benthic invertebrates samples were collected (Town Beach and One tree) where four samples were taken.

From each water sample the concentration of the following elements were analysed:

- Dissolve Organic Carbon (DOC): This consists of organic molecules. Organic carbon compounds are the result of decomposition processes from dead organic matter (e.g. phytoplankton and mangrove). DOC can support the growth of microorganisms, like heterotrophic bacteria.
- Iron (Fe): The low concentration of iron present in the ocean is typically oxidized iron, Fe<sup>3+</sup>. This oxidized Fe forms inert iron oxides and hydroxides and is thus essentially not bioavailable to photosynthetic organisms. On the other hand, Fe<sup>2+</sup>, the reduced form of iron, is more soluble and thus more biological reactive, but rapid oxidation to Fe3<sup>+</sup> would ostensibly make the presence of Fe<sup>2+</sup> nearly impossible. Therefore iron could be an important limiting nutrient in the oceans and in coastal zones (Martin et al. 1994). Organically chelated iron can promote the exponential growth of *Lyngbya majuscula* (Ahern et al. 2008).
- Nitrate/nitrite (NOx): NOx is one form of dissolve inorganic nitrogen (DIN). In the natural nitrogen cycle, bacteria (e.g. cyanobacteria) convert nitrogen to nitrate, which

is taken up by primary producers and incorporated into tissues. It is the primary form of nitrogen responsible for primary production in the oceans because it is more abundant in marine systems than NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> (Gruber et al 2008). Nitrate and nitrite compounds are very soluble in water and quite mobile in the environment.

- Ammonia (NH<sub>3</sub>): NH<sub>3</sub> is another form of dissolve inorganic nitrogen (DIN). Together with ammonium (NH<sub>4</sub><sup>+</sup>), it is thought to be the preferred source of fixed nitrogen for phytoplankton, because it can be easily assimilated with little energy expenditure (Zehr and Ward, 2002). Ammonia is the most reduced form of nitrogen and is found in water where dissolved oxygen is lacking. When dissolved oxygen is readily available, bacteria quickly oxidize ammonia to nitrate through a process known as nitrification. Other types of bacteria produce ammonia as they decompose dead plant and animal matter. High ammonia concentrations can stimulate excessive aquatic production and indicate pollution (EPA 2012).
- Total Nitrogen (TN): In marine ecosystems nitrogen is usually a limiting element for biological productivity. Nitrogen can promote the exponential growth of *Lyngbya majuscula* (Ahern et al. 2008).
- Total Phosphorus (TP): In marine ecosystems phosphorus is also often a limiting element for biological productivity. Phosphorus can promote the exponential growth of *Lyngbya majuscula* (Ahern et al. 2008).

In sediment samples the concentration of the following compounds were analysed:

- Iron
- Nitrite-nitrate
- Ammonium
- Total Nitrogen

## - Total Phosphorus

Water quality was assessed against the ANZECC/ARMCANZ (2000) water quality guidelines for slightly disturbed estuaries of tropical Australia. These guidelines provided water and sediment quality guidelines for protecting a range of aquatic ecosystems, from freshwater to marine (ANZECC/ARMCANZ 2000). The primary objective of the guidelines is to "maintain and enhance the 'ecological integrity' of ecosystems" (ANZECC/ARMCANZ 2000). The guidelines indicate that since "no data are available for tropical estuaries or rivers of Western Australia, a precautionary approach should be adopted when applying default trigger values to these systems".

All the nutrient concentration analyses were carried out in the Chemistry Centre of Western Australia.

#### Sediment grain size

There is often a close association between benthic invertebrates and sediment grain size. Community composition (Ysebaert and Hermanand 2002 MEPS) and biomass of intertidal benthic macroinvertebrates (Riccardi and Bourget 1999) have been related to sediment grain size.

Sediment samples for sediment grain size were collected in December 2010, February and April 2011. Sediment samples for organic matter analysis were collected in October and December 2010, February, April and December 2011, January, February, March and April 2012. Samples were collected from the same locations sampled for benthic invertebrates and nutrient. Three to four replicates were collected in each sampling site.

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After drying the sample for 24 hours at 60 °C, it was sieved using a stack of sieves with mesh

sizes of 63, 125, 212, 355, 500 and 1000 µm. The following categories of were used:

≥1000 µm: Very coarse sand.

500 µm: Coarse sand.

212-355 µm: Medium sand.

125 µm: Fine sand.

63 µm: Very find sand and

< 63 µm: Silt.

Sediment organic matter content

Sediment organic matter (SOM) in estuarine and coastal areas is of great significance to the

biogeochemical cycling of carbon, nutrients cycles and primary productivity. The sources of

SOM are diverse and include detrital matter, inputs from land, seagrasses, epiphytes,

mangroves, macroalgae, microphytobenthos and phytoplankton.

Sediment samples for sediment organic matter content were collected in October and

December 2010, February, April and December 2011, January, February, March and April

2012. Samples were collected from the same locations sampled for benthic invertebrates and

nutrient. Three to four replicates were collected in each sampling site.

The samples were stored in labelled bags and frozen for subsequent analysis. In the

laboratory, samples were dried for 24 h at 60°C and weighted Dry Mass (DM). The dried

sample was then combusted at 500 °C for 2 h in a muffle furnace. Ash Free Dry Mass

(AFDM, being a measure of the organic content of the sample) was determined as the

difference between the DM and the remaining ash.

## Diversity and abundance of benthic invertebrates

To evaluate whether the diversity and abundance of benthic invertebrates varied in the presence and absence of *Lyngbya majuscula*, samples of benthic invertebrates were taken from Town Beach and One Tree in November 2009, February, October and December 2010, February, April and December 2011 and January, February, March and April 2012.

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 17 years, was followed. Two stations were defined at each site, one 150 m offshore and the second 250 m offshore, along a transect perpendicular to the coast. At each station four samples were taken, each one consisting of six cores of 10.3 cm diameter (Plate 4). Therefore each sample represented a sampled of 0.05 m<sup>2</sup> sediment and each station sampled a surface area of 0.2 m<sup>2</sup>.









**Plate 4**. Some of the volunteers that participated in the macroinvertebrate sampling. Pictures show the material used for benthic sampling, cores and sieves.

The samples were initially passed through a 1 mm sieve on the beach to remove most of the coarser sediment and then through a 0.5 mm sieve (Plate 5).





**Plate 5**. Sieving samples on site though the 0.5 mm sieve in Town Beach.

The samples were then labelled and preserved in 70% ethanol and returned to the laboratory for processing. All samples were processed using a stereomicroscope (10 x 22). All individual species were removed, identified to family level, and abundance of each family recorded. Family level taxonomy has been shown to be an appropriate level to detect changes in soft bottom assemblages (Bertasi et al. 2009).

Diversity was determined using the Shannon-Wiener diversity index.

## Direct observations of shorebirds foraging behaviour

The quality of different feeding habitats for shorebirds was measured directly using prey selection studies and intake rates (Piersma et al. 1993, Goss-Custard et al. 1995). Random individuals from the species Bar-tailed Godwit *Limosa lapponica* feeding actively at low tide were selected and observed for 3 minute periods through a Leica Televid 25 x 60 telescope

during daylight (Plate 6), recording: number of successful prey captures (feeding rates) and type and size of prey caught.

The observations were made when *Lyngbya majuscula* was present (February 2010, February 2011) and when it was absent in the Bay (November 2009, October 2010) in two locations, Town Beach and One Tree. An average of  $35 \pm 12$  observations were taken per site and date.

Biomass intake rate was calculated by multiplying the number of a prey size classes taken per unit time by the ash free dry mass (AFDM) of the size class of the invertebrate species collected from benthic sediments. The Bar-tailed Godwit, which is one of the most common shorebird species in Roebuck Bay, was selected among the other species because of its size, bigger than other shorebird species, making the direct observations easier.

**Plate 6.** Observations of shorebirds feeding behaviour using a telescope from One Tree beach, February 2011.



## Stable isotopes analysis

Stable isotope analysis is widely used in ecological studies (Hobson 2005, Pain et al. 2004). The signature of stable isotopes in plants and animals varies according to a range of biological and geographic factors such as habitat type, trophic level of diet and geographic

location (Romanek et al. 2000). The stable isotopes signature of the consumer reflects that of its prey, so that animals carry an isotopic registry in their tissues of what they have consumed and of the overall environmental conditions. More specifically, nitrogen stable isotopes exhibit stepwise enrichment with trophic transfers through food webs and can thus provide trophic-level information, and so are frequently used as dietary markers (Hobson and Clark 1992). Stable isotopes have also assisted explaining patterns of movement of consumers between habitats (Hansson et al. 1997, Hadwen et al. 2007) as well as indicating nutrient enrichment (e.g. Twefik et al 2007, Piñón-Gimate et al. 2009, Teichberg et al. 2010). Carbon isotopes differ between different plant types, reflecting different photosynthetic pathways, and thereby enable discrimination between broad food sources, which may reflect habitat types (Romanek et al. 2000).

Measurements of  $\delta^{15}N$  and  $\delta^{13}C$  stable isotope proportions in metabolically active tissues of shorebirds, in muscle of macroinvertebrate and in whole plankton organisms as well as seagrass and mangrove leaves were therefore employed in establishing the trophic resources used as well as the type and condition of habitat.

- Primary producers: Phytoplankton was collected with plankton nets (Plate 7) of different mesh sizes and concentrated onto Whatmann glass-fibre filter (GF/C) with the help of a vacuum pump.

**Plate 7**. Plankton net used during the study for plankton collection in Roebuck Bay, WA.



Diatoms were collected and concentrated off surficial sediments. The top 1-2 millimetres of surface sediment was collected at low tide by careful scraping, targeting areas were a 'sheen' of diatoms was evident on the surface. In the laboratory the wet sediment was covered by GF/C filters and a bright light placed over the filter papers. The diatoms were then attracted to the light and migrated from the sediment and into the filter paper, where they were collected (Compton et al. 2008). Mangrove (three leafs from three individual trees per sampling) and seagrass leafs (three leaves from several individuals of Halophila ovalis and Halodule uninervis) were collected from each site. Epiphytes were remove from the seagrass leaves using a blade and the help of a stereomicroscope. Lyngbya majuscula was collected from Town Beach site in February 2010. The filters and other samples were frozen immediately for storage. Phytoplankton was acid-washed to remove carbonates. All samples were rinsed with distilled water, dried and ground to a fine powder prior to stable isotope analysis. It was not possible to collect samples of degraded seagrass and Lyngbya (detritus). However, it is unclear whether  $\delta^{13}$ C of living seagrass is generally distinct from the  $\delta^{13}C$  of seagrass detritus (Belicka et al. 2012). Therefore fresh seagrass and Lyngbyasamples were used as a carbon source proxy of their respective detritus.

Particulate Organic Material (POM): Particulate Organic Material was collected from each sampling site by elutriation and sieving. Sediment at each site was placed in a bucket of water and resuspended to suspend organic matter, and then passed through a sieve to capture suspended organics and omit heavier coarse sediment. Samples were frozen immediately for subsequent analysis in the laboratory. All samples were acid-washed to remove carbonates, rinsed with distilled water, dried and ground to a fine powder prior to stable isotope analysis.

- Primary consumers of different functional feeding groups were collected. Suspension feeders such as some species of bivalves' species and deposit feeders such as polychaete worms and other groups such as crabs and bivalves were collected using a PVC-pipe corer, sieve and frozen immediately for subsequent analysis in the laboratory. Determination to species level was carried out where possible.

For crabs, three to five individuals were pool together and only the muscle from the legs was used for the stable isotope analysis. In the case of bivalves, one to three individuals were pool together. The bivalve foot was selected for the analysis. All samples were rinsed with distilled water, dried and ground to a fine powder prior to stable isotope analysis. When the whole animal was used for the analysis, the gut content was removed by depurating the organism in filtered sea water for 24 h.

Shorebirds species: The Bar-tailed Godwit *Limosa lapponica* was selected given it is reported as feeding on large worms. The Great Knot *Calidris tenuirostris* was selected as a mollusk-eater, whose populations are in decline (Rogers et al. 2009). The Red-necked Stint *Calidris ruficollis* was selected as potential predator of small crustaceans and worms and also as potential grazer of biofilm (Kuwae et al. 2012). The diet of the Red-necked Stint in Roebuck Bay is completely unknown. Approximately 30 individuals from the selected species were captured in each season (wet and dry) with cannon nets in the programmed ringing campaigns carried out annually by the Global Flyway Network and the Australasian Waders Study Group in Roebuck Bay (Minton 2006). Most of the captured individuals were ringed, weighed and morphological measurements taken. A small amount of blood (≤ 10% total bird blood volume; Tsahar et al. 2008) was extracted from the brachial vein of each individual for isotope analysis (Plate 8).

Blood samples were frozen immediately for subsequent analysis. Blood samples were freezedried and ground to a fine powder with a lancet prior to stable isotope analysis. To complement the stable isotope results, a study of the diet was carried out through direct observation of birds feeding (see *Direct Observations of Shorebirds Foraging Behaviour*).

All samples for stable isotope analysis were taken from two locations, Town Beach and One Tree. The exception was blood samples from shorebirds, which were collected from different locations, depending on suitable conditions for cannon netting and where shorebirds aggregated. Sampling was conducted twice in the wet season (February 2010 and February 2011) and twice in the dry season (November 2009 and October 2010). In February 2010 no blood samples were collected for stable isotopes analysis as the Global Flyway Network and the Australasian Waders Study Group banding program was not occurring.



**Plate 8.** Blood extraction from a Great Knot in Roebuck Bay, WA. It is possible to observe the drop of blood from the brachial vein and the capillary tube with the blood sampled.

With the exception of shorebirds blood samples, all samples for stable isotope analysis were dried to a constant weight at 60 °C, homogenized with a ball grinding mill into a fine powder and weighed to the nearest 0.001 g prior to analysis. The analysis was carried out at the West Australian Biogeochemistry Centre (WABC), University of Western Australia with a

continuous flow system consisting of a Delta V Plus mass spectrometer connected to a Thermo Flush 1112 via Conflo IV (Thermo-Finnigan/Germany). The value is expressed as the deviation in parts per thousand from the standard, using the following relationship  $\delta$ :  $\delta$  % = ([Rm – Rst] / Rst) x 1000, where R is the quantity of heavy isotope, divided by the quantity of the lighter isotope, both for the sample 'm', and the standard 'st'. Normalization was performed using international standards provided by IAEA:  $\delta^{13}$ C - NBS22, USGS24, NBS19, LSVEC and for  $\delta^{15}$ N - N1, N2, N3 and laboratory standards. Results had a precision of 0.1‰ (1 SD) for  $\delta^{15}$ N and 0.1‰ (1 SD) for  $\delta^{13}$ C.

### **Data Analysis**

## - Univariate analysis

Normality and homoscedasticity were tested (Shapiro-Wilk and Levene's test respectively) for each variable prior to statistical analysis to confirm the data met the assumptions of the relevant tests. When normality of the data was not achieved a  $\log_{10}(x+1)$  transformation was applied (Sokal and Rohlf 1995). The differences in abundance, richness and diversity of invertebrates, prey captured per minute and intake rates of shorebirds and concentrations of total nitrogen (N\_tot) between sampling dates (fixed factor) and among sites (fixed factor) were analysed using a two-way ANOVA test. Differences in prey captured per minute in relation to 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.

When the variables did not meet the assumptions of parametric analysis after transformation, non-parametric test were use. Differences in *Lyngbya* biomass between sampling dates, sites and distance to shoreline and differences in nutrient concentrations among sampling dates and sites were analysed using Kruskal-Wallis test.

Values are presented as means  $\pm$  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).

## - Multivariate analysis

Multivariate analyses were performed using the PRIMER package v 6 (Plymouth Routines in Multivariate Ecological Research; Clarke and Gorley 2006) to investigate differences in macroinvertebrates assemblages across sites and sampling periods (and later seasons/years), and relationships with physico-chemical characteristics from each site. The PRIMER package was developed for multivariate analysis of marine fauna samples and has been applied extensively on environmental studies. Types of analysis to be applied to the data included:

1. Describing pattern amongst the assemblage data using cluster and ordination techniques based on Bray-Curtis similarity matrices. The clustering technique uses a hierarchical agglomerative method where samples of similar assemblages are grouped and the groups themselves form clusters at lower levels of similarity. A group average linkage was used to derive the resultant dendrogram. Ordination of data was by Multi-Dimensional Scaling (MDS) (Clarke & Warwick 2001), with ordinations depicted as two- or three-dimensional plots based on the site by site similarity matrices.

- 2. For any groups found in (1) or selected *a priori* (i.e. before/during/after 2010 *Lyngbya* bloom), Analysis of Similarity (ANOSIM) effectively an analogue of the univariate ANOVA was conducted to determine if groups were significantly different from one another in ordination space. The ANOSIM test statistic reflects the observed differences between groups (e.g. sites) with the differences amongst replicates within the groups. The test is based upon rank similarities between samples in the underlying Bray-Curtis similarity matrix. The analysis presents the significance of the overall test (Significance level of sample statistic), and significance of each pairwise comparison (Significance level %), with degree of separation between groups (R-statistic), where R-statistic >0.75 = groups well separated, R-statistic >0.5 = groups overlapping but clearly different, and R-statistic >0.25 = groups barely separable. A significance level <5% = significant effect/difference (p < 0.005).
- 3. The relationship between the environmental and biotic data were assessed with the BEST routine to calculate the minimum suite of physico-chemical parameters that explain the greatest percent of variation in benthic fauna assemblage data (i.e. the parameters which most strongly influence the species ordination)
- 4. Permutational multivariate analysis of variance (PERMANOVA) was used (two-factor crossed design) to determine whether there were any significant difference in the benthic fauna assemblages between sampling collection date in relation with the *Lyngbya majuscula* bloom of 2010 and among sites (Anderson 2001a, b, McArdle and Anderson 2001, Anderson and ter Braak 2003, Anderson et al. 2008).

# - SIAR V4

To provide a description of the main primary producers that support some of the food webs in Roebuck Bay, the importance of each potential source was evaluated. Since

macroinvertebrates showed differences in  $\delta^{15}N$  and  $\delta^{13}C$  values between sites, and were well separated within functional feeding groups and primary producers showed also differences in  $\delta^{15}N$  and  $\delta^{13}C$  values, we used a Bayesian multiple source isotope mixing model (SIAR package -stable isotope analysis in R (Parnell et al. 2008, 2010; Jackson et al. 2009); R Development Core Team, Vienna). A Bayesian approach can be used to estimate diet composition in underdetermined systems (i.e. more diet sources than isotopes) and can directly account for uncertainty and variation in the isotopic composition and elemental concentrations of sources and consumer tissues, as well as trophic enrichment factors (Parnell et al. 2008, 2010; Jackson et al. 2009). Resulting models identify a range of solutions for the proportion of each food item, with the median of these solutions representing the maximum likelihood. These models require an estimate of fractionation factors, which is the expected difference in isotope ratio between dietary items and consumers (i.e. shorebird-blood). A fractionation factor of -0.41%  $\pm$  1.14 and 2.52%  $\pm$  2.5 for herbivores from Vander Zanden and Rasmussen (2001) was used for  $\delta^{15}N$  and  $\delta^{13}C$  respectively for each trophic transfer.

To provide a description of shorebirds' diet (Bar-tailed Godwit, Great Knots and Red-necked Stints) in Roebuck Bay, the importance of each potential source was evaluated. Since macroinvertebrates showed differences in  $\delta^{15}N$  and  $\delta^{13}C$  values between sites and were well separated within functional feeding groups, we used also a Bayesian multiple source isotope mixing model (SIAR package -stable isotope analysis in R (Parnell et al. 2008, 2010; Jackson et al. 2009); R Development Core Team, Vienna). A fractionation factor of 2.9‰  $\pm$  0.3 and  $1.3\% \pm 0.3$  was used for  $\delta^{15}N$  and  $\delta^{13}C$  respectively (Evans Ogden et al., 2003).

## - Partial Least Squares Regression

To investigate which variables correlated most with *Lyngbya* biomass of each sampling site or wet season, two Partial Least Squares Regression (PLS) models were developed in Statistica 7.0 (StatSoft. Inc., Tulsa, Oklahoma, USA). PLS regression is particularly suited for studies where the matrix of predictors has more variables than observations, and when there is multi colinearity among variables (Carrascal et al. 2009).

Due to the study conditions (the bloom with the highest biomass occurred during the pilot study, wet season 2009-2010-, when nutrient concentrations were not measured) it was not possible to develop a single model which included all potential factors. This also should serve as a precaution note in relation with the results of the models. Because there are no water or sediment quality data for the major bloom event, the results of the models should be consider as indicative factors of *Lyngbya* blooms in Roebuck Bay.

The PLS<sub>climatological</sub> model correlated mean *Lyngbya* biomass of the area found between Port of Broome and Town Beach for February of three years (2010, 2011 and 2012) with climatological variables (Table 1). The climatological variables were chosen in accordance with previous studies (Johnson et al 2010) and personal observations as potential factors that may affect the initiation of *Lyngbya* blooms and therefore, *Lyngbya* biomass. It was considered that the occurrence of a *Lyngbya* bloom was affected by conditions that occurred prior to the bloom (before or during *Lyngbya* reached its maximum biomass). Therefore climatological conditions of the first part of the wet season (December to February) were selected. Data on sea water temperature in Broome were unavailable for December 2009 to February 2010 and therefore an analysis of the climatological influences on *Lyngbya* biomass focused upon the effect of three main factors: air temperature, rainfall and solar radiation (global solar radiation) (table 2). All climate data used within this study were available from the Australian Bureau of Meteorology (BOM). Climate data were collected from the nearest weather station at Broome Airport (station #003003). Global solar exposure is the total

amount of solar energy falling on a horizontal surface (BOM). Mean biomass of *Lyngbya* each February was analysed against the climatological variables. The PLS<sub>climatological</sub> model was conducted in a step-wise manner that allowed the removal of variables that did not contribute to the model, enabling the strongest possible PLS model to be created.

**Table 1.** Climate and environmental factors used in Partial Least Squares (PLS) regression analysis of *Lyngbya majuscula* biomass changes at Roebuck Bay, North Western Australia, (2010 to 2012).

Factor	Elements used in PLS climate regression				
Air temperature	Mean air temperature December to February, mean air temperature				
(°C)	December, mean air temperature January, Mean air temperature February				
	Total rainfall December to Febr uary, total rainfall December, t otal rainfall				
Total rainfall (mm)	January, total rainfall February				
Solar radiation	Mean solar radiation December to February, mean solar radiation December,				
(MJ/m <sup>2</sup> )	mean solar radiation January, m ean solar radiation February				

**Table 2.** Climate data in Roebuck Bay, North Western Australia (wet seasons 2009-2010, 2010-2011 and 2011-2012). Data from the Australian Bureau of Meteorology (BOM) (station #003003).

Year	Month	Total monthly rain fall (mm)	Air mean monthly max temperature (ºC)	Air mean monthly min temperature (ºC)	Solar exposure (MJ/m²)
2009	12	179.4	33.9	26.6	26.9
2010	1	140	33	26.6	26.4
2010	2	6.4	34.1	27.8	29
2010	12	85.8	33.6	27.3	27.6
2011	1	449.2	32.2	25.1	20.7
2011	2	275	32.2	25.5	21.7
2011	12	0.4	34.5	27.7	29.8
2012	1	255.2	33.2	25.9	28.4
2012	2	147.8	34.2	25.5	29.8

The PLS<sub>nutrients</sub> model correlated mean wet season (December to April) *Lyngbya* biomass in the sampling sites (POB, TB, DC, CS, FP and OT) with sediment and water quality variables as well as dissolve organic carbon (DOC) and sediment grain size (% mud) (see Table 3). The variables were chosen in accordance with previous studies (Ahern et al. 2008) and personal observations of potential factors that may affect *Lyngbya* biomass. As it has been indicated above, The PLS<sub>nutrients</sub> model was conducted in a step-wise manner that allowed the removal of variables that did not contribute to the model, enabling the strongest possible PLS model to be created.

**Table 3.** Sediment and water factors used in Partial Least Squares (PLS) regression analysis of *Lyngbya majuscula* biomass changes at Roebuck Bay, North Western Australia, Australia (2010 to 2012).

Factor	Elements used in PLS <sub>nutrients</sub> regression					
	Mean total Nitrogen (mg/Kg), mean ammonium (mg/Kg), mean nitrate +					
Nutrients in	nitrite fraction (mg/Kg), mean total phosphorus (mg/Kg), mean organic					
sediment	phosphorous (mg/Kg), mean iron (mg/Kg)					
	Mean dissolved organic carbon (mg/L), mean total Nitrogen (mg/L), mean					
Nutrients in water	ammonia (mg/L), mean nitrate + nitrite fraction (mg/L), mean total					
	phosphorus (mg/L), mean iron (mg/L)					
Sediment grain sized	% Mud (particle size < 63 μm)					
Sediment organic	% Organic material					
material						



Lyngbya majuscula in Roebuck Bay, blue crab, fan worm and starfish (Photos: Tom de Silva)

# RESULTS

# **RESULTS**

# Mapping coverage and biomass of Lyngbya majuscula

(Objective 1)

Lyngbya majuscula in Roebuck Bay was present during the study in the area contained between Port of Broome and Fall Point, with coverage changing over time (Figures 4 to 12). No Lyngbya was found in any other parts of the Bay. The biomass was temporally and spatially variable. However two hot spots of Lyngbya biomass were found in the Bay in the three years study. One was localised between Port of Broome and Town Beach and the other between Dampier Creek and Fall Point (see Figures 4, 6 and 10). Lyngbya biomass peaked each wet season, but the bloom that occurred in the wet season of 2009-2010 was the most severe of the three studied (Figure 4). Lyngbya biomass showed significant differences among sites (H = 363.94, d.f. = 7, p < 0.001, Figures 4 to 12) and distance from the shoreline (H = 83.3, d.f. = 7, p < 0.0001, Figure 13).



Figure 4. Extension and intensity of Lyngbya majuscula bloom in February 2010 after a visual reconnaissance of the study area.



Figure 5. Extension and intensity of Lyngbya majuscula bloom in December 2010 from collected samples.



Figure 6. Extension and intensity of Lyngbya majuscula bloom in February 2011 from collected samples.

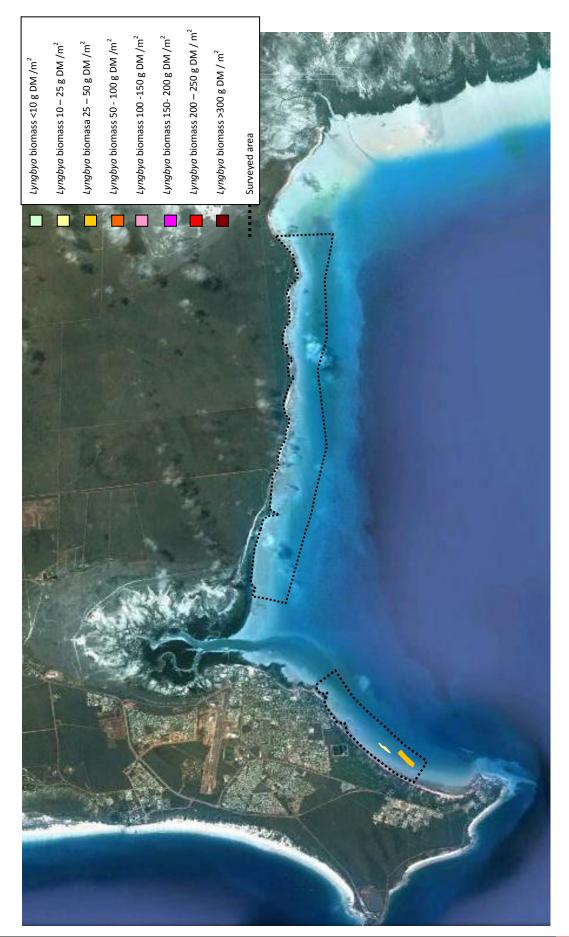


Figure 7. Extension and intensity of Lyngbya majuscula bloom in April 2011 from collected samples.



Figure 8. Extension and intensity of Lyngbya majuscula bloom in December 2011 from collected samples.



Figure 9. Extension and intensity of Lyngbya majuscula bloom in January 2012 from collected samples.



Figure 10. Extension and intensity of Lyngbya majuscula bloom in February 2012 from collected samples.

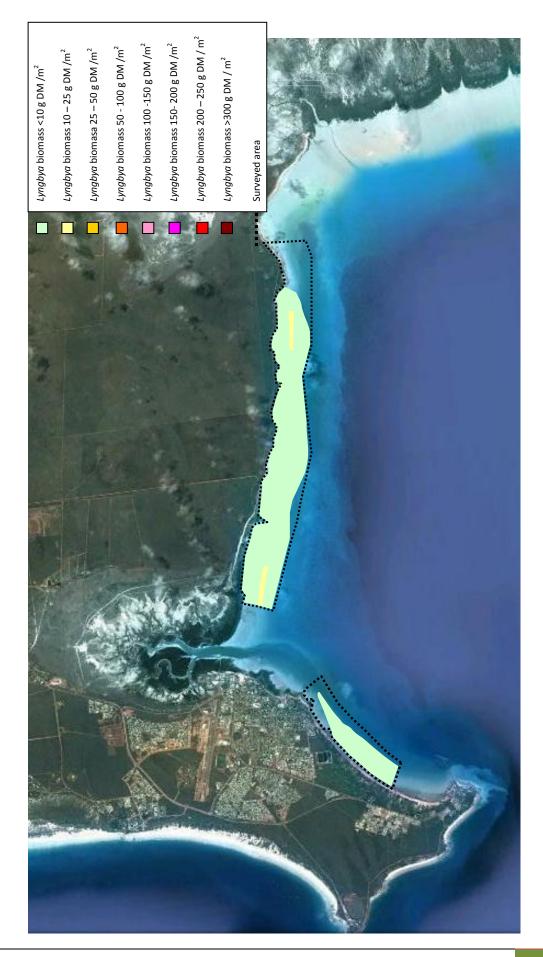


Figure 11. Extension and intensity of Lyngbya majuscula bloom in March 2012 from collected samples.

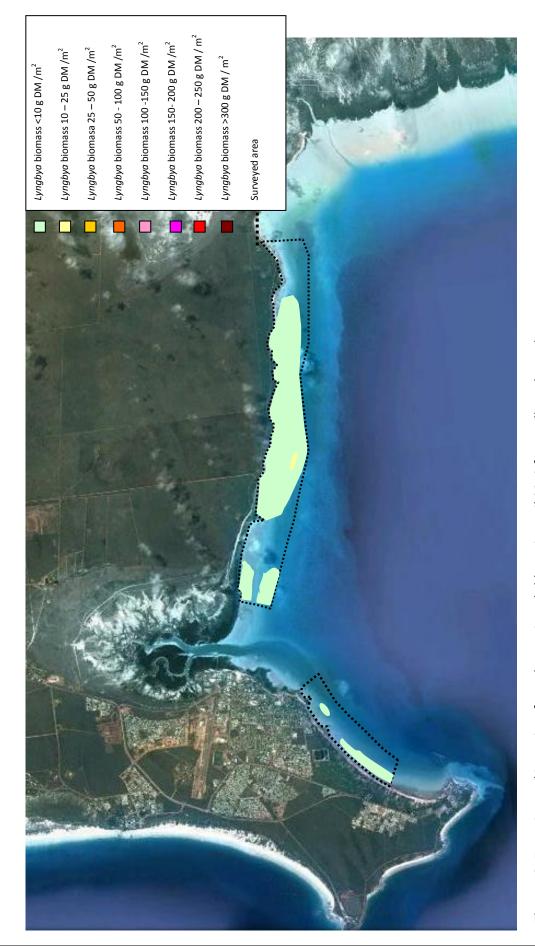
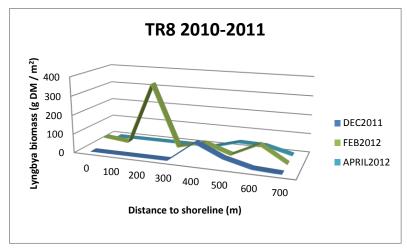
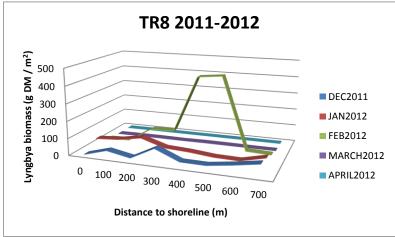


Figure 12. Extension and intensity of Lyngbya majuscula bloom in April 2012 from collected samples.

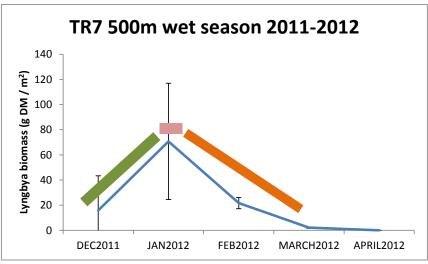
**Figure 13.** Changes in *Lyngbya majuscula* biomass a long transect eight in different dates within two wet seasons in Roebuck Bay, WA.

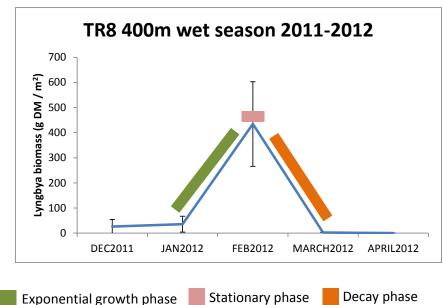




The development of the *Lyngbya* bloom followed a similar pattern to that observed in Moreton Bay (Ahern et al. 2007, Johnson et al. 2007). First there was an exponential growth that last probably from November/December to February, then there was a stationary phase that considered in February, followed by the decay phase, which was characterised by a decline in *Lyngbya* biomass from February to April (Figure 14). The exact timing of each phase varied among sites and across years (Figure 14).

Figure 14. Changes in Lyngbya majuscula biomass during the different phases of the bloom at 500 m and 400 m from the shoreline in transects 7 and 8 respectively. Mean ± SD.





Overall, in areas where Lyngbya blooms were active, the minimum biomass of *Lyngbya* found throughout the study was 0.08 g DM /m<sup>2</sup> with a maximum of 2076.4 g DM / m<sup>2</sup>. During the peak of the bloom the range of biomass varied from 1.01 to 2076.4 g DM / m<sup>2</sup>. The average biomass during the peak of the bloom (February) in the wet seasons 2009-2010, 2010-2011 and 2011-2012 in the area between Port of Broome and Town Beach was 331.57  $\pm$  152.07, 81.21  $\pm$  33.09 and 76  $\pm$  30.41 respectively. The average biomass during the peak of the bloom in the wet seasons 2010-2011 and 2011-2012 in the whole area (from Port of

Broome to One Tree) was  $22.31 \pm 8.91$  and  $41.19 \pm 9.37$  respectively. No data was available for the wet season 2009-2010 between Dampier Creek and One Tree.

## Potential triggers of Lyngbya blooms in Roebuck Bay

### (Objective 1)

The biomass of *Lyngbya* was positively correlated with rainfall and solar exposure in December and average temperature in January. 89 % of the variability in biomass of *Lyngbya* observed between wet seasons was explained by the first component of the PLS<sub>climate</sub> model (Table 4), with the main factor being total rainfall in December. Conversely, biomass of *Lyngbya* was negatively correlated with the ammonium and organic phosphorous concentration in the sediment. The second component of the PLS<sub>nutrients</sub> model explained the 61% of the variability in *Lyngbya* biomass among sites (Table 4) during the study period.

**Table 4.** Results of the Partial Least Squares regression analysis (PLS) (final models following stepwise analysis) carried out with *Lyngbya majuscula* mean biomass in Roebuck Bay, WA as response variable of three wet seasons (February 2010-2012; PLS<sub>climate</sub> model) and six sites (December to April 2011 and 2012; PLS<sub>nutrients</sub> model). Predictor variables were climatological factors and sediment nutrient concentrations. W COMP1 and W comp2: weights of each variable in the first and second PLS component. R<sup>2</sup>: proportion of the variance in the response variable accounted for by each component of the PLS. The nutrients predictor variables were included log-transformed in the model.

	W COMP1	W COMP2	Reg. Coeff
PLS <sub>CLIMATE</sub>			
Total rainfall December	0.59	-0.11	0.26
Solar exposure December	-0.45	0.64	11.93
January temperature	0.67	0.75	281.9
$R^2$	0.89	0.11	
PLS <sub>NUTRIENTS</sub>			
Ammonium	-0.51	-0.94	-83.30
Organic phosphorous	-0.86	0.34	-83.20
$R^2$	0.39	0.61	

#### **Sediment nutrient concentrations**

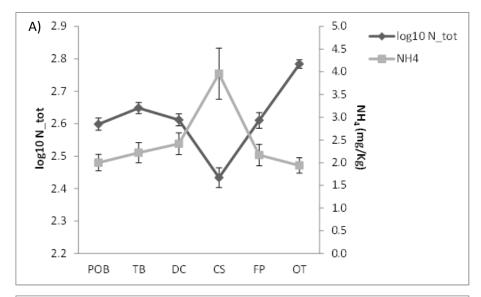
## (Objective 2)

There were significant differences among sites for concentrations of all nutrients in sediments (Table 5, Figure: 15 A) and B)). The lower concentrations were generally found at Camp Site while the highest nutrient sediment concentrations were recorded at One Tree. The exception was ammonium, which had maximum concentration at Camp Site (Figure: 15 A) and B)). There were significant differences in concentration of iron (Fe), ammonium (NH<sub>4</sub>-N and total nitrogen (N\_tot) in sediments among sampling dates (Table 5, Figure: 16 A) and B)). No significant differences were found for extractable phosphorous (P (HCO3)) or total phosphorous (P\_tot) (Table 5, Figure: 16 A) and B)). Nitrate (NO<sub>3</sub>\_N) was bellow detection limits in most samples and therefore was not included in statistical analysis.

**Table 5.** Kruskal-Wallis non-parametric and ANOVA to test for significant differences in sediment nutrient concentrations by sampling date and site in Roebuck Bay, NWA.

	DATE				SITE		
	d.f.	Н	р		d.f.	Н	р
Fe	7		66.18	<0.0001	5	44.44	<0.0001
$N_NH_4$	7		80.54	<0.0001	5	22.67	<0.001
P extractable	7		11.41	0.12	5	124.65	<0.001
P_tot	_ 7		5.03	0.66	5	132.1	<0.001
	d.f.	F		р	d.f.	F	р
log <sub>10</sub> N_tot	7	25	5.56491	<0.0001	5	101.861	<0.0001

Figure 15. Spatial variation in sediment concentration of A) log<sub>10</sub> total nitrogen, ammonium, B) total phosphorous and extractable phosphorus in Roebuck Bay, NWA. Mean ± SE.



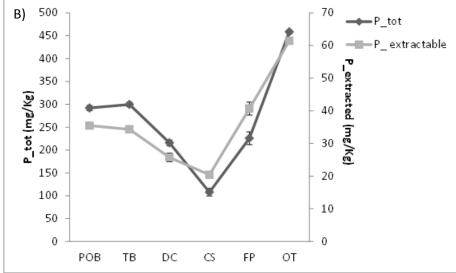
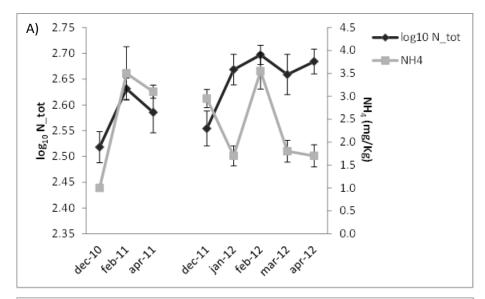
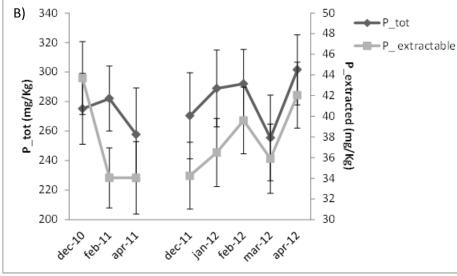


Figure 16. Temporal variation in sediment concentration of A) log 10 total nitrogen, ammonium, B) total phosphorous and extractable phosphorus in Roebuck Bay, NWA. Mean ± SE.





#### Water nutrient concentrations

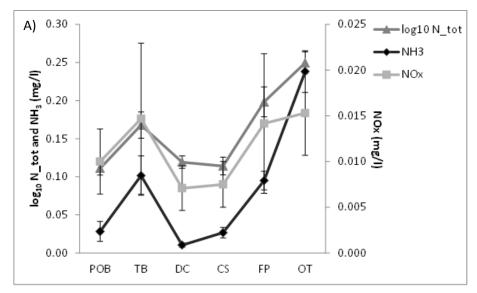
#### (Objective 2)

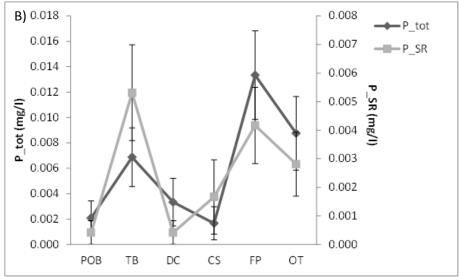
There were no significant between-site differences in the concentrations of dissolve organic carbon (DOC), nitrites + nitrates (N\_NOx) and or iron (Fe), while there were significant differences for all other nutrients (Table 6, Figure: 17 A)-C)). The lower concentrations generally occurred in Dampier Creek and Camp Site, with highest concentrations of nutrients in water from Town Beach, Fall Point and One Tree (Figure: 17 A)-C)). Analysis detected significant between-sampling occasion differences in concentrations of all nutrients in water except iron (Fe) and ammonia (N\_NH<sub>3</sub>), which showed no significant differences (Table: 6, Figure: 18 A)-C)).

**Table 6.** Kruskal-Wallis non-parametric and ANOVA to test for significant differences in water nutrient concentrations by sampling occasion and site in Roebuck Bay, NWA.

			DATE				SITE	
	d.f.	Н		р	d.f.	Н		р
DOC	7		21.19	0.0035	5		6.78	0.24
Fe	7		5.06	0.65	5		2.6	0.76
$N_NH_3$	7		12.64	0.08			87.14	<0.0001
N_NOx	7		83.41	<0.001			2.34	0.8
P_SR	7		45.13	<0.0001	5		14.3	0.014
P_tot	7		44.52	<0.0001	5		16.23	0.006
	d.f.	F		р	d.f.	F		р
log <sub>10</sub> N_tot	7	7.	129027	<0.0001	5	2	26.9832	<0.0001

Figure 17. Spatial variation in water concentration of A) log<sub>10</sub> total nitrogen, ammonia, nitrite + nitrate, B) total phosphorous, soluble reactive phosphorus and C) dissolve organic carbon in Roebuck Bay, NWA. Mean ± SE.





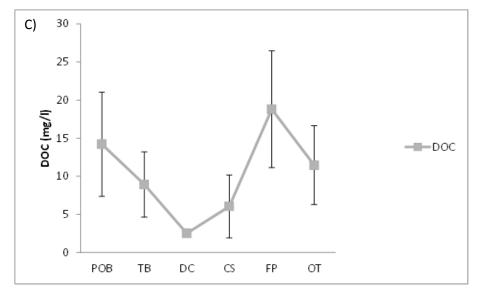
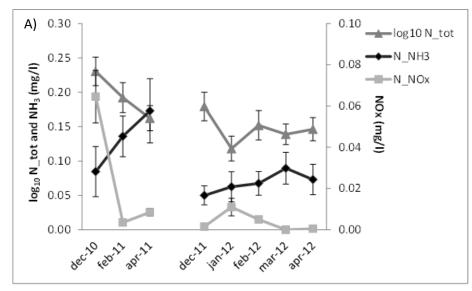
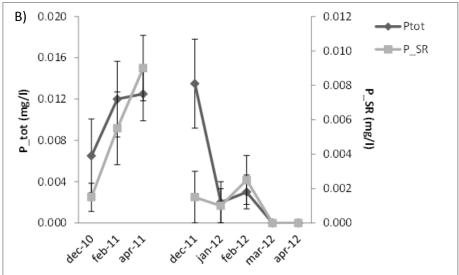
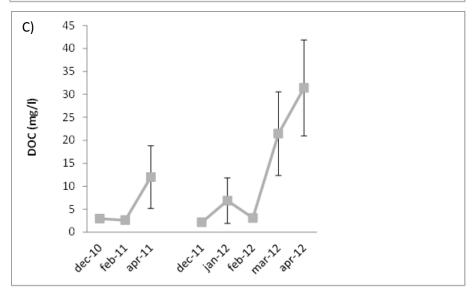


Figure 18. Temporal variation in water concentration of A) log<sub>10</sub> total nitrogen, ammonia, nitrite + nitrate, B) total phosphorous, soluble reactive phosphorus and C) dissolve organic carbon in Roebuck Bay, NWA. Mean ± SE.







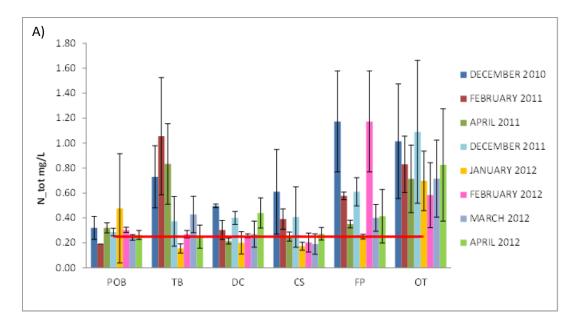
In relation to water quality, the ANZECC/ARMCANZ (2000) trigger values for slightly disturbed estuaries of tropical Australia within are presented in table 7.

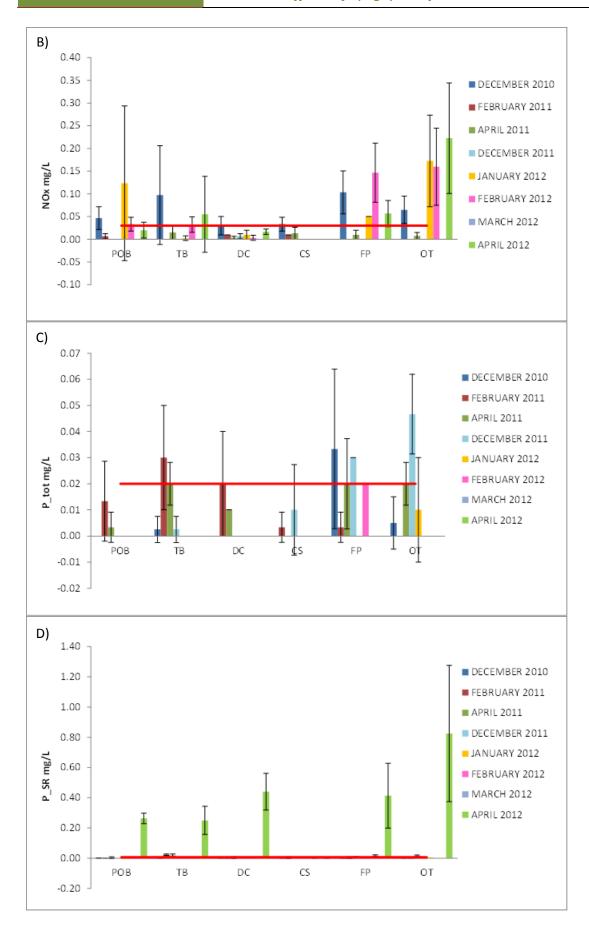
**Table 7.** Trigger values for chemical stressors and toxicants for slightly disturbed estuaries of tropical Australia within the ANZECC/ARMCANZ (2000).

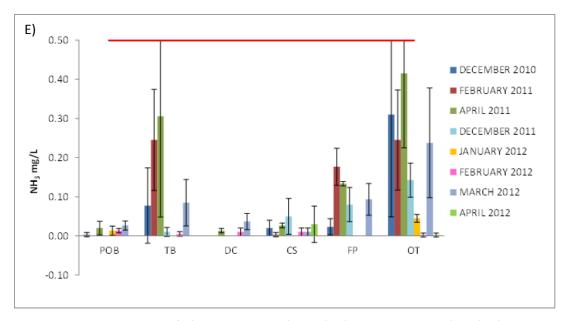
=	P_tot (mg/l)	P_SR (mg/l)	N_tot (mg/l)	NOx (mg/l)	NH₃ (mg/l)³
Estuaries	0.02	0.005	0.25	0.03	0.5
Marine Inshore	0.015	0.005	0.1	0.008	

<sup>&</sup>lt;sup>a</sup>NH<sub>3</sub> is a non-metallic inorganic toxicant. The trigger value for NH<sub>3</sub> for slightly disturbed systems is 0.91 mg/l (ANZECC/ARMCANZ, 2000). However Roebuck Bay is an ecosystem of high conservation/ecological value (RAMSAR site since 1990 and future Marine Park). It implies that the management goal should be no change in biodiversity (ANZECC/ARMCANZ, 2000).

Concentrations of total nitrogen (N\_tot), nitrites + nitrates (NOx), total phosphours (P\_tot) and soluble reactive phosphorus (P\_SR) were in exceedance of current ANZECC/ARMCANZ (2000) default trigger values (Figure 19 A)-D)). Ammonia (NH<sub>3</sub>) was below the ANZECC/ARMCANZ (2000) trigger values for toxicants (Figure 19 E)).



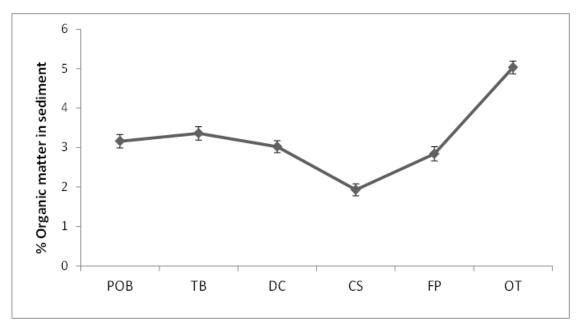




**Figure 19.** Concentration of A) total nitrogen (N\_tot), B) nitrite + nitrate (NOx), C) total phosphorous (P\_tot), D) soluble reactive phosphorus (P\_SR) and E) ammonia (NH $_3$ ) in water from five sampling stations over 2010, 2011 and 2012 in Roebuck Bay, NWA. ANZECC/ARMCANZ (2000) trigger value for each parameter is indicated by the red line. Data showed as mean  $\pm$  SD.

#### Sediment organic matter content

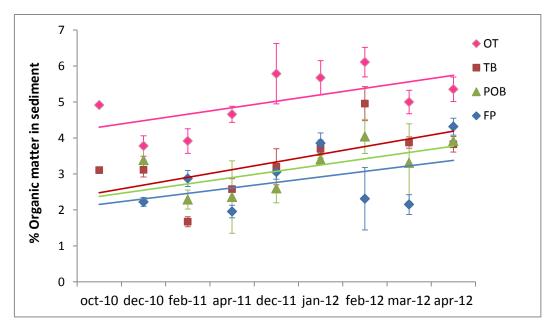
Organic matter (OM) content in sediments had significant temporal (F = 22.45, df = 7, p < 0.00001) and spatial differences (F = 121.07 df = 5, p < 0.00001, Figure 20) in Roebuck Bay. The interaction factor was also significant (Date x Site: F = 6.41, df = 35, p < 0.00001). The highest content was found at One Tree (OT) and the minimum was found at Camp Site (CS) (Figure 20). There was a significant increase in OM in One Tree, Fall Point (FP), Town Beach (TB) and Port of Broome (POB) over the study (Table 8, Figure 21), but with no significant change over time at Dampier Creek (DC) and Camp Site (Table 8, Figure 21).



**Figure 20.** Spatial variation in sediment organic matter content in Roebuck Bay, NWA, from October 2011 to April 2012. POB: Port of Broome; TB: Town Beach; DC: Dampier Creek; CS: Camp Site; FP: Fall Point; OT: One Tree. Values represent mean ± SE.

**Table 8.** Pearson r correlation of sediment organic matter against date for different sites in Roebuck Bay, NWA, from October 2011 to April 2012. POB: Port of Broome; TB: Town Beach; DC: Dampier Creek; CS: Camp Site; FP: Fall Point; OT: One Tree. \*\*\*: p < 0.05; n.s.: non-significant, p > 0.05.

	r	p
OT	0.74	***
TB	0.7	***
POB	0.47	***
DC	0.12	n.s.
CS	0.27	n.s.
FP	0.44	***



**Figure 21.** Temporal changes in sediment organic matter content in Roebuck Bay, NWA, from October 2011 to April 2012 in Port of Broome (POB), Town Beach (TB) and One Tree (OT). Values represent mean ± SE. Values for October 2010 are from one sample.

# Sediment grain sized

The sediment of the northern section of Roebuck Bay from Port of Broome to One Tree is characterised mostly by fine sand. Sediment grain size separated this section of the Bay (sampling area) into three different areas: the western part of the Bay (Port of Broome and Town Beach) was characterised by fine sand sediments, the northern intertidal area (Dampier Creek to Fall Point) was also characterised by fine sand but with a significant percentage (around 15%) of medium size sand. And the eastern part of the Bay (One Tree) was composed mostly by mud and fine sand (Figure 22).

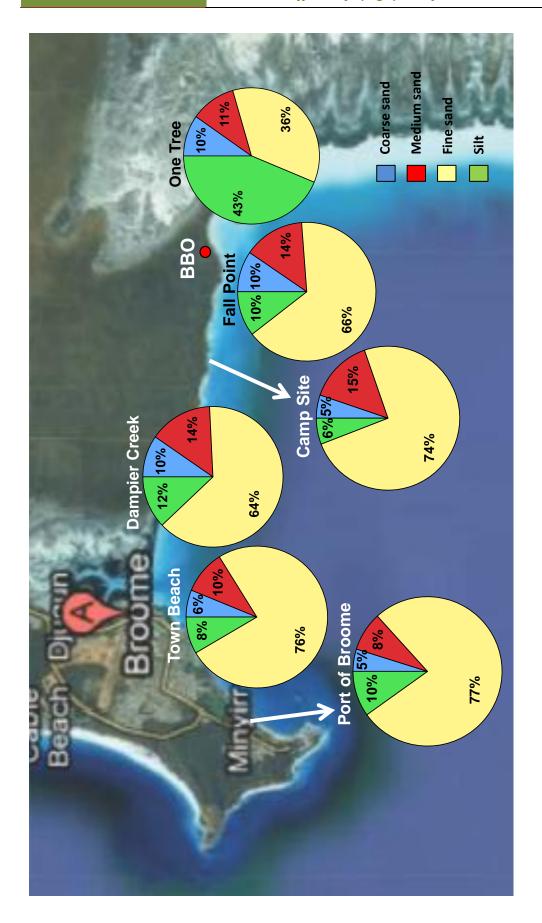


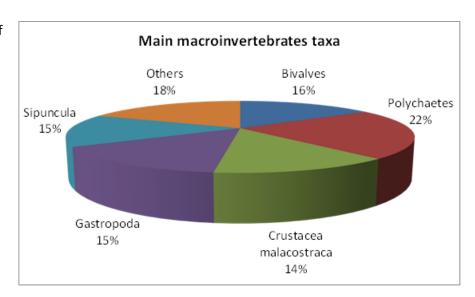
Figure 22. Sediment grain sized composition of different sites of Roebuck Bay, NWA.

## Temporal and spatial variation of benthic invertebrates' diversity and abundance

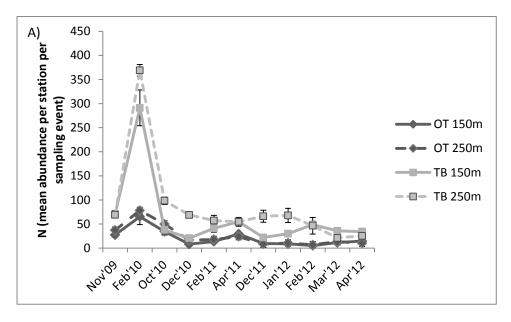
#### (Objective 3)

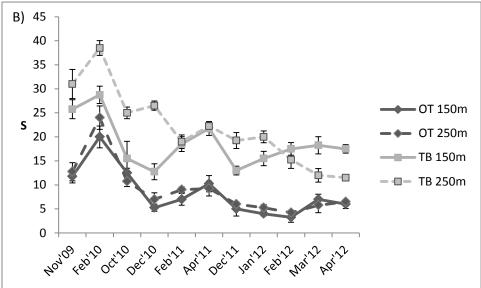
A total of 8825 benthic invertebrates were collected during the study. Of these, 11 main invertebrate taxa were identified. Polychaete worms and bivalves compromised more than 38% of the total fauna (Figure 23). Although sipunculids represented 14.7% of the total fauna, in February 2010 more than 40% of the fauna were sipunculids and almost 24% were gastropods.

Figure 23. Proportion of the dominant benthic macroinvertebrate taxa found at Roebuck Bay, NWA from November 2009 to April 2012.



There were significant spatial and temporal differences in the abundance (N) and species richness (S) of benthic macroinvertebrates', with high abundances and species richness at Town Beach and One Tree in February 2010 (Figure 24, Table 9). Abundance and species richness were consistently higher at Town Beach than One Tree (Figure 24). Distance to shoreline significantly affected the abundance and species richness at both sites (Figure 24, Table 9).



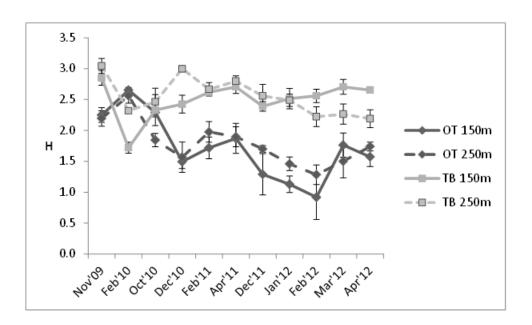


**Figure 24.** Macrobenthic invertebrate A) abundance and B) species richness in Town Beach (light grey) and One Tree (dark grey), Roebuck Bay, NWA from November 2009 to April 2012. Solid line indicates the stations situated 150 m off the shoreline and the broken line indicates the stations situated 250 m off the shoreline. Values are mean ± SE.

Diversity of benthic invertebrates was significantly different among sampling dates and between sites, with a maximum at One Tree and a minimum at Town Beach in February 2010 (Figure 25, Table 9). The interaction factor between Site, Date and Distance from shoreline was also significant (Figure 25, Table 9). At Town Beach, in February 2010, the station

situated 150 m from the shore on bare sand had a significant lower diversity than the station situated 250 m from the shore on seagrass (Figure 25).

Species richness then increased from February 2010 at all sites and stations, and the reduced diversity observed at Town Beach reflected a large increase in the abundance of several taxa, specially snails and sipunculids. These taxa reached abundances of more than 1500 and 3000 individuals per square meter respectively in February 2010 (Figure 25 E) and F)).



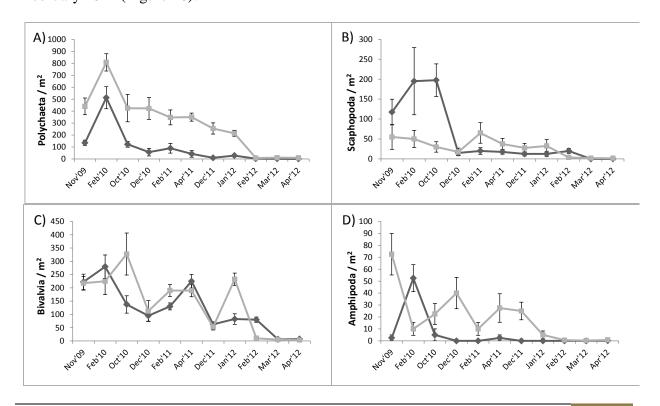
**Figure 25.** Shannon-Wiener diversity index (H) for macrobenthic invertebrate samples of Town Beach (light grey) and One Tree (dark grey), Roebuck Bay, NWA from November 2009 to April 2012. Solid line indicates the stations situated 150 off the shore and the broken line indicates the stations situated 250 m off the shore. Values mean ± SE.

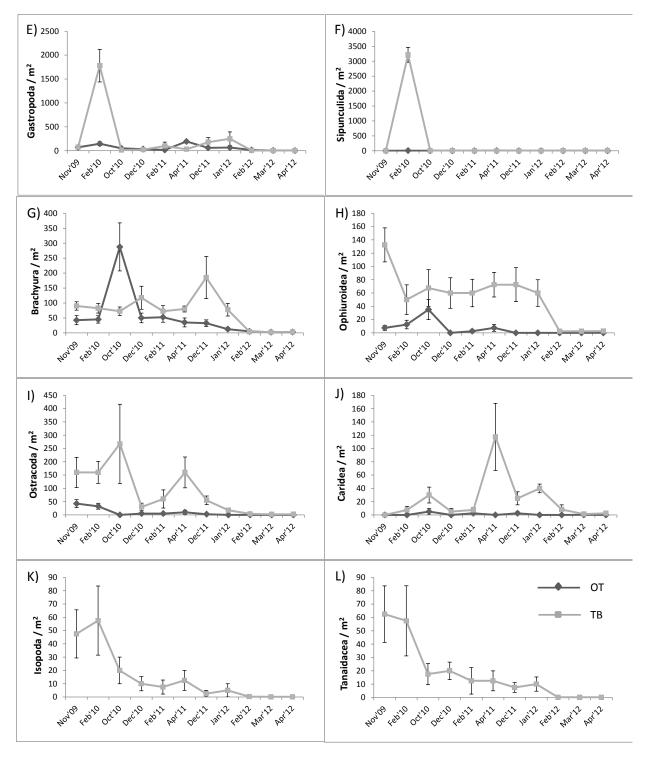
**Table 9.** Three-factor ANOVA on the effects of sampling date, site and distance from shore on macrobenthic invertebrate abundance (N), species richness (S) and diversity (H) in Roebuck Bay, NW Australia.

		S			N			Н	
	F	d.f.	p	F	d.f.	p	F	d.f.	p
Site	486.99	1	***	510.70	1	***	256.27	1	***
Distance	9.37	1	**	27.05	1	***	1.58	1	0.21
Date	28.62	10	***	64.42	10	***	8.50	10	***
Site x Distance	0.01	1	0.92	2.16	1	0.14	0.05	1	0.82
Site x Date	5.50	10	***	8.01	10	***	10.89	10	***
Date x Distance	2.73	10	**	5.30	10	***	1.74	10	0.08
Site x Distance x Date	1.90	10	0.05	2.55	10	*	2.27	10	*

p < 0.05 \*; p < 0.01 \*\*; p < 0.001 \*\*\*

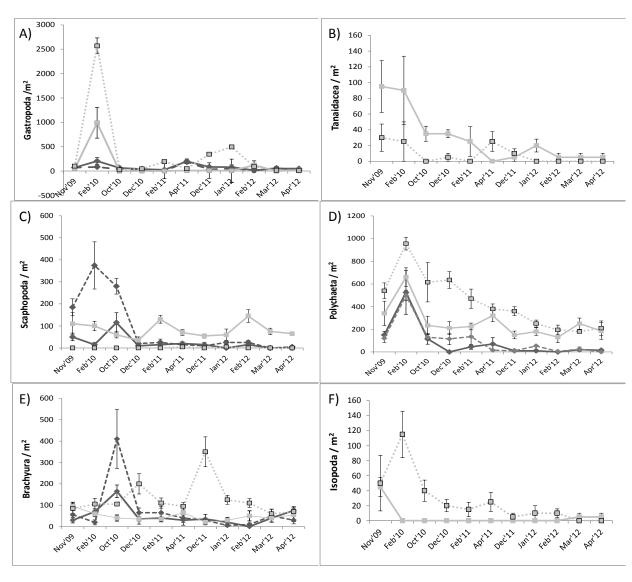
All taxa showed significant differences in abundance over time (Table 10). Several dominant taxa had significant increases in abundance increases in February 2010, among them polychaetes (Polychaeta), tusk shells (Scaphopoda), bivalves (Bivalvia) and amphipods (Amphipoda) at One Tree and polychaetes, snails (Gastropoda) and sipunculids (Sipunculida) at Town Beach (Figure 26 A)-F), Table 10). Other taxa showed an increase in abundance in October 2010, for example at One Tree crabs (Brachyura) and brittle stars (Ophiuroidea) and at Town Beach seed shrimps (Ostracoda) and bivalves (Figure 26 G)-I) and C), Table 10). Seed shrimps and shrimps (Caridea) also showed a significant increase in abundance in April 2011 at Town Beach, while amphipods showed a significant decrease in abundance in February 2010 (Figure 26 I), J) and D), Table 10). Isopods (Isopoda) and tanaids (Tanaidacea) were recorded only at Town Beach, where they showed a significant decrease in abundance since February 2010 (Figure 26 F) and J), Table 10). Except for bivalves, tusk shells and snails all had significant differences in abundance between sites (Figure 26, Table 10). At both sites several taxa declined in abundance or were no longer recorded after February 2012 (Figure 26).

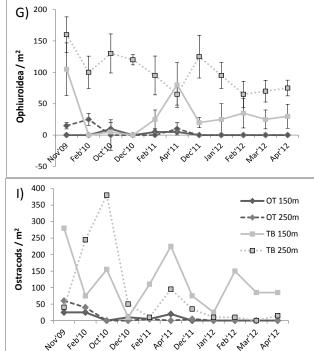




**Figure 26.** Abundance of dominant benthic macroinvertebrate taxa at Town Beach (light grey) and One Tree (dark grey), Roebuck Bay, NWA from November 2009 to April 2012. A) Polychaeta, B) Scaphopoda, C) Bivalvia, D) Amphipoda, E) Gastropoda, F) Sipunculida, G) Brachyura, H) Ophiuroidea, I) Ostracoda, J) Caridea, K) Isopoda and L) Tanaidacea. Values mean ± SE.

Distance from shoreline also affected the abundance of benthic macroinvertebrates (Figure 27, Table 10). Although some taxa were significantly more abundant close to the shore, such as snails at both sites and tanaids and tusk shells at Town Beach (Figure 27 A), B) and C), most taxa had significantly higher densities further from the shore, such as polychaetes, crabs, isopods and brittle stars at both sites and tusk shells at One Tree (Figure 27 D), E), F), G) and C)). The patterns in abundance of some taxa such as polychaetes, bivalves and snails were similar at both stations, 150 and 250 m off the shoreline, at One Tree and Town Beach. However, the abundance of other taxa followed different patterns depending of the distance from the shore, especially in the first three months of the study, such as tusk shells atn One Tree and seed shrimps, brittle starts and isopods at Town Beach (Figure 27 C), I), G) and F)).





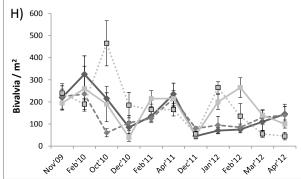


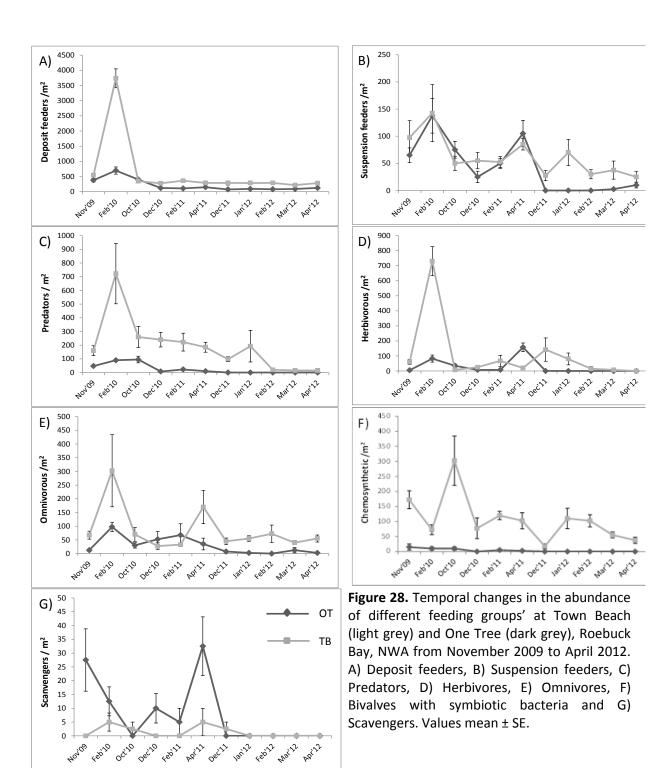
Figure 27. Abundance of dominant benthic macroinvertebrate taxa at different distances from the shore at Town Beach (light grey) and One Tree (dark grey), Roebuck Bay, NWA from November 2009 to April 2012. A) Gastropoda, B) Tanaidacea, C) Scaphopoda, D) Polycaheta, E) Brachyura, F) Isopoda, G) Ophiuroidea, H) Bivalvia and I) Ostracoda. Solid lines are the stations situated 150 m from the shore and broken lines are the stations situated 250 m from the shore. Values mean ± SE.

# Temporal and spatial variation of benthic invertebrates' feeding groups abundance

## (Objective 3)

The abundance of benthic macroinvertebrate feeding groups showed significant temporal effects. At both Town Beach and One Tree deposit feeders, suspension feeders and predators showed an increase of abundance in February 2010 (Figure 28 A)-C), Table 11). At Town Beach herbivores and omnivores also showed an increase in abundance in February 2010, while bivalves with symbiotic bacteria (chemosynthetic) showed a significant increase in October 2010 (Figure 28 D)-F), Table 11). Scavengers were not found at either site since January 2012 (Figure 28). From December 2011 onwards at One Tree several feeding groups, among them chemosynthetic bivalves, suspension feeders, herbivores and predators, either

disappeared or their numbers decreased significantly (Figure 28). All feeding groups showed differences in abundance between One Tree and Town Beach (Figure 28, Table 11).



Distance from shoreline and the interaction factor also showed significant effects on the abundance of feeding groups (Figure 29, Table 11). Suspension feeders and deposit feeders showed a significant increase in abundance at both stations at Town Beach and One Tree in February 2010. Omnivores also showed significant increases at all stations except at the station closest to the shore at Town Beach. There was a significant increase in the abundance of predators in February 2010 at the seagrass station (205 m offshore) at Town Beach while chemosynthetic bivalves increased at the same station in October 2010.

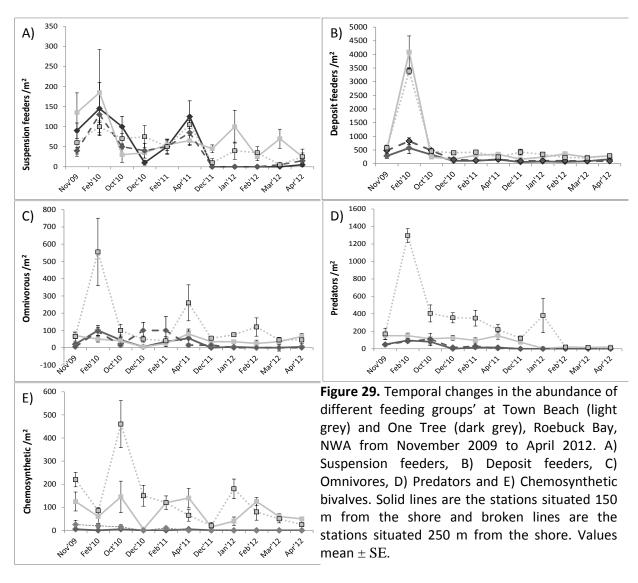


Table 10. Factorial ANOVA and Kruskal-Wallis test results of sampling date, site and distance to coast effects on major benthic macroinvertebrate taxa abundance in Roebuck Bay, NWA.

•		Site			Date			Distance	ce		Site x Date	Date	Sit	x Dis	Site x Distance	Ω	Date x Distance	tance	Site	x Date	Site x Date x Distance
	ц	d.f.	Д	ц	d.f.	d.f. p	ц	d.f.	d	Ц	d.f.	d	ц	d.f.	р	Ц	d.f.	d	口	d.f.	Д
ivalvia	1.98	-	0.16	9.27	9.27 10	<0.00001	0.08	1	0.77	3.6	10	0.0003	0.12	10	0.73	1.55	10	0.13	3.56	10	<0.001
chyura	32.7	_	<0.00001		4.21 10	<0.0001	22.31	-	<0.00001	4.64	10	<0.0001	18.13	10	<0.0001	2.40	10	0.012	1.97	10	0.04
phopoda	0.02	_	Scaphopoda 0.02 1 0.89		10	<0.00001	48.21	_	<0.00001	11.05	10	<0.00001	217.98	10	<0.00001	3.00	10	0.002	3.66	10	<0.001
/chaeta	406.07	_	<0.00001		10	25.88 10 <0.00001	22.7	_	<0.00001	4.33	10	<0.0001	2.48	10	0.120000	2.77	10	0.004	0.1	10	0.450000
phipoda	64.38	_	<0.00001		10	< 0.00001	0.67	_	0.42	7.29	10	<0.00001	3.92	10	0.05	3.43	10	<0.001	2.2	10	0.02
tropoda	0.06	-	0.8	20.11 10	10	< 0.00001	13.47	_	<0.001	10.36	10	<0.00001	46.15	10	<0.00001	1.79	10	0.07	1.76	10	0.08
iuroidea	232.96	-	<0.00001		10	3.01 10 0.002	89.57	_	<0.00001	2.60	10	0.007	45.24 10	10	<0.00001	3.12	10	0.001	1.76	10	0.07
	$\chi^{2}$	d.f.	$\chi^2$ d.f. p	$\chi^2$	d.f.	$\chi^2$ d.f. $p$	$\chi^{2}$	d.f.	d												
dea	59.15	1	<0.00001		10		0.45	-	0.5												
Ostracoda	61.47	1	61.47 1 < 0.00001	22.42 10	10	0.01	4.46	-	0.04												
ipunculida	7.87	_	0.005	59.25	10	<0.00001	0.065	_	8.0												
Tanidacea*				19.64	10	19.64 10 0.03	10.67	_	0.001												
Isopoda*				27.23	10	0.002	13.82	_	<0.001												

\*Taxa only found in Town Beach

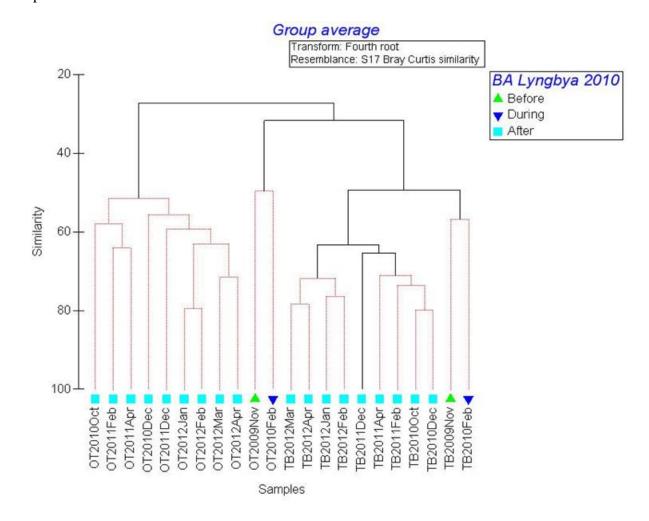
Table 11. Factorial ANOVA and Kruskal-Wallis test results of sampling date and site effects on major benthic macroinvertebrate feeding groups abundance in Roebuck Bay, NWA.

		Site			Date	e		Distance	ıce		Site x Date	Date	S	ite x D	Site x Distance	ı	ate x D	Date x Distance	Site	Date 3	Site x Date x Distance
	ц	d.f.	d	ц	d.f.	F d.f. p	ц	d.f.	Ь	Ц	d.f.	Ь	ц	d.f.	Ь	Ц	d.f.	Ь	ц	d.f.	Ь
Chemosynthetic	355.00	1	355.00 1 <0.00001	7.51	10	.51 10 <0.00001	10.18	1	0.002	3.59	10	<0.001	1.83	10	0.18	4.22	10	< 0.0001	3.15	10	0.0010
Deposit feeders		-	220.84 1 <0.00001	55.33	10	55.33 10 <0.00001	16.65	-	<0.001	6.73 10	10	<0.00001	0.25	0.25 10	0.62	2.78	2.78 10	0.004	1.55	10	0.13
Suspension feeders		_	25.56 1 <0.00001	21.41	10	<0.00001	2.61	_	0.1	4.27	4.27 10		1.65	10	0.2	2.10	2.10 10	0.03	2.38	10	0.01
Predators	296.51	_	296.51 1 <0.00001	38.16	10	38.16 10 <0.00001	39.82	_	< 0.00001	6.74	10	< 0.00001	22.75	10	<0.00001	4.54	10	< 0.0001	3.37 10	10	<0.001
Omnivorous	10.99	-	66.01 1 <0.00001	7.33	10	7.33 10 <0.00001	15.76	_	0.002	2.77 10	10	0.004	9.62	10	0.003	2.19	2.19 10	0.02	2.08 10	10	0.03
	$\chi^2$	d.f.	d	$\chi_z$	d.f.	$\chi^2$ d.f. p	$\chi^{z}$	d.f.	d												
Herbivorous	5.92	1	0.015	55.76	11	55.76 11 <0.00001		1	90.0												
Scavengers	9.28	-	9.28 1 0.0023 39.79 11 <0.00001	39.79	11	<0.00001		1	0.7												

# Effects of Lyngbya majuscula and distance to shore on benthic macroinvertebrates community

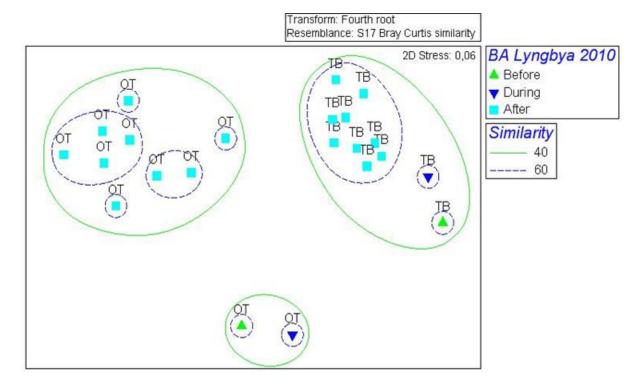
#### (Objective 3)

Cluster analysis of macroinvertebrate community assemblages showed a clear separation of sites and of samples collected before, during and after the *Lyngbya majuscula* bloom of February 2010 (Figure 30). This was supported by MDS ordination of fourth root transformed family abundance data using Bray-Curtis similarity matrix which showed a clear separation of sampling location, and of samples collected before, during and after *Lyngbya majuscula* bloom of 2010 (Figure 31). The resultant ordination was achieved with an optimum solution in three dimensions and a stress of 0.15.



**Figure 30.** Cluster analysis of benthic macroinvertebrate abundance data (fourth root transformed) using Bray-Curtis similarity index showing similarities among the samples based on their community

size structure (NB because the number of replicate samples was high, average values per distance to coast and sampling date are presented to simplify the Figure).

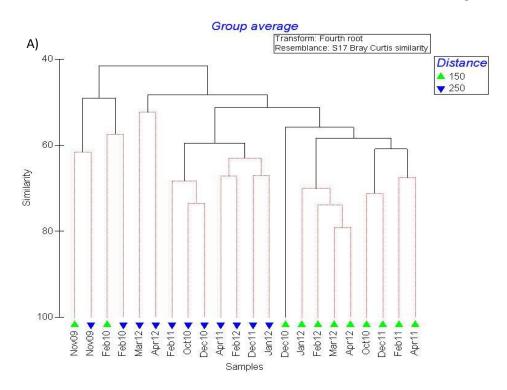


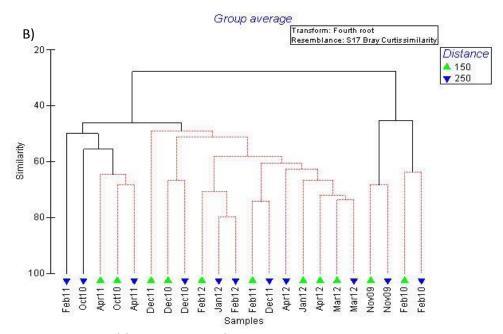
**Figure 31.** Non-metric multidimensional scaling ordination (MDS) of the group-average Bray–Curtis clusters obtained, with fourth root transformation, using the abundance of the different macroinvertebrate families of the four stations within the two localities. All samples were significantly different in their composition (PERMANOVA P < 0.05) (NB because the number of replicate samples was high, average values per distance to coast and sampling date are presented to simplify the Figure).

ANOSIM detected a significant difference in macroinvertebrate assemblage composition from samples before, during and after the Lyngbya bloom in February 2010 (R-statistic = 0.60, p = 0.001) and between sites differences (R-statistic = 0.87, p = 0.001). PERMANOVA similarly detected a significant difference in assemblage composition between sites (PERMANOVA pseudo-F = 1.88, df = 2, p = 0.001), and sampling occasions (before, during and after Lyngbya majuscula bloom of 2010) (PERMANOVA pseudo-F = 3.61, df = 1, p = 0.001), with a significant interaction effect (PERMANOVA pseudo-F = 7.616, df = 2, p = 0.001).

The BEST routine showed that percentage of mud and concentration of extractable phosphorus in the sediment explained the most variation in the assemblage data, accounting for 75% of variation.

CLUSTER analysis presented a clear separation within samples from Town Beach in relation with their distance to the shore (Figure 32 A)), however, this separation did not exist in samples from One Tree (Figure 32 B)). ANOSIM showed a significant effect due to distance to shore on the macroinvertebrate assemblage from Town Beach (R-statistic = 0.50, p = 0.001), however such an effect was not found at One Tree (R-statistic = -0.006, p = 0.61).





**Figure 32.** Cluster analysis of fourth root transformed benthic macroinvertebrate abundance data using Bray-Curtis similarity index showing similarities among samples based on their community composition for A) Town Beach and B) One Tree (NB because the number of replicate samples was high, average values per distance to coast and sampling date are presented to simplify the Figures).

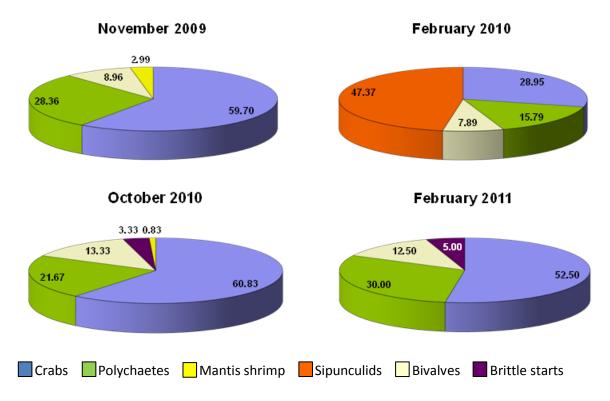
#### Shorebird foraging behaviour and diet. Effects of Lyngbya blooms

# (Objective 3)

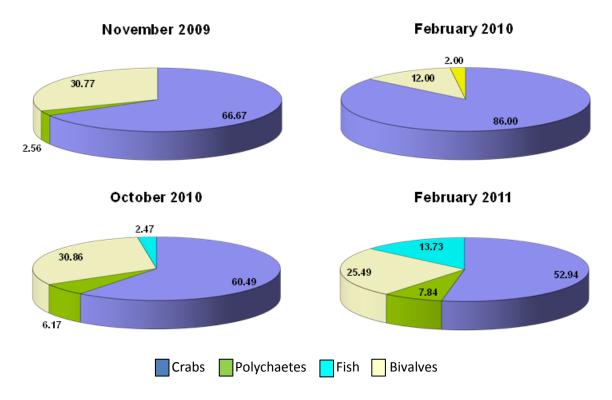
Direct observations of the foraging behaviour of Bar-tailed Godwits (*Limosa lapponica*) showed that the main prey at both Town Beach and One Tree were crabs (Figures 33 and 34). The second most common prey varied in relation with relative benthic invertebrate abundances at each site; at Town Beach it was polychaetes (Figure 33) which was an abundant benthic invertebrate (Figure 26) while at One Tree it was bivalves (Figure 34), that was also an abundant invertebrate within depredable prey size. There were no marked changes in the diet of Bar-tailed Godwits between the dry and wet seasons. However, there was a significant change in the diet of Bar-tailed Godwits at Town Beach when *Lyngbya* was present in February 2010 compare with other periods. In November 2009, October 2010 and February 2011 the main prey of Bar-tailed Godwits were crabs, in February 2010 the main

prey was sipunculids (Figure 33). At One Tree there was not a noticeable change in diet in February 2010 compared with surveys before and after this date.

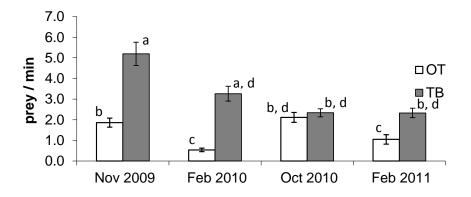
The number of prey captured per minute (feeding rate) was significantly affected by the feeding site (F = 115.32, df = 1, p < 0.00001), date (F = 16.85, df = 3, p < 0.00001), with a significant interaction factor (F = 12.92, df = 3, p = 0.00001) (Figure 35). At One Tree there was an increase in the feeding rate in the dry season and a decrease in the wet season (Figure 35). At Town Beach on the other hand, the peak feeding rate was in November 2009 followed by a decrease in capture success (Figure 35).



**Figure 33.** Percentage of captured and ingested prey observed in the diet of Bar-tailed Godwits feeding in Town Beach, Roebuck Bay, NWA, in the dry and wet seasons of 2009-2010 and 2010-2011.



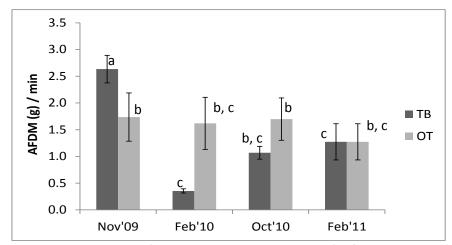
**Figure 34.** Percentage of captured and ingested prey observed in the diet of Bar-tailed Godwits feeding in One Tree, Roebuck Bay, NWA, in the dry and wet seasons of 2009-2010 and 2010-2011.



**Figure 35.** Mean feeding rates  $\pm$  SE (prey captured per minute) of Bar-tailed Godwits in at Town Beach (TB) and One Tree (OT) in Roebuck Bay, NWA in the dry and wet seasons of 2009-2010 and 2010-2011. Bars with the same letter are not significantly different (Tukey's range test).

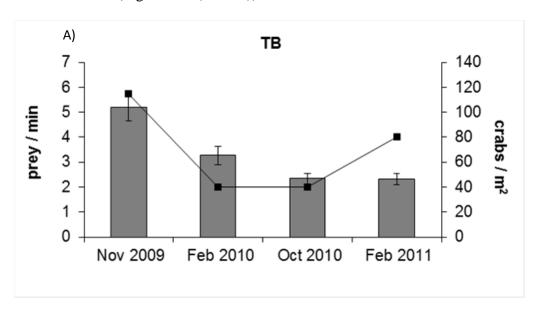
ANOVA on biomass of prey consumed per minute (intake rate) by Bar-tailed Godwits detected significant effects for sampling date (F = 15.49, df = 3, p < 0.00001) and the interaction factor (F = 7.45, df = 3, p = 0.0001), while was no significant difference in intake

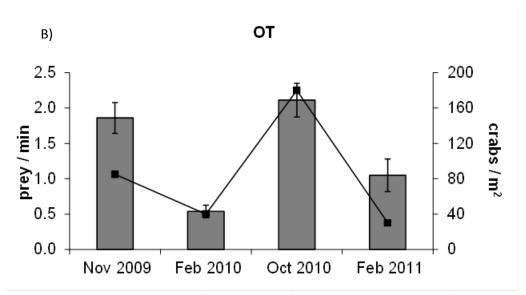
rates across sampling sites (F = 2.00, df = 1, p = 0.15) (Figure 36). At One Tree the intake rates obtained by the godwits were similar throughout the study, whilst at Town Beach intake rates in November 2009 were significantly higher than all other survey times (Figure 36).



**Figure 36.** Mean intake rates ± SE (biomass ingested per minute) of Bar-tailed Godwits in Town Beach (TB) and One Tree (OT) in Roebuck Bay, NWA in the dry and wet season of 2009-2010 and 2010-2011. Bars with the same letter are not significantly different (Tukey's range test).

The sentinel crabs (*Macrophthalmus sp.*) are the most common crab in the intertidal flats of Roebuck Bay. There was a relationship between Bar-tailed Godwits feeding behaviour and the abundance of their main prey, sentinel crabs. The feeding rates of the Bar-tailed Godwits at Town Beach and One Tree followed a similar temporal pattern as the one defined by the abundance of this crab (Figure 37 A) and B)).





**Figure 37.** Relationship between mean feeding rates of Bar-tailed Godwits mean feeding rates (grey bars) ± SE at A) Town Beach and B) One Tree with abundance of sentinel crabs (black line).

#### Primary producers stable isotopes analysis

#### (Objective 2)

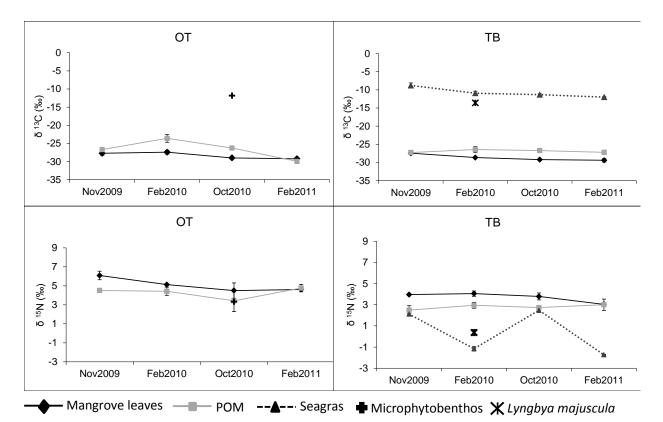
Samples of different primary producers (i.e. mangroves leaves, seagrass, microphytobenthos, Lyngbya and plankton and coarse particulate organic matter (POM)) were collected where possible in each site in each sampling occasion. Plankton were collected using fine-mesh nets from open water in the middle of the Bay. Concentrations of the isotopes  $^{13}$ C and  $^{15}$ N in the samples of plankton size  $\geq 250 \mu m$  were under detection limits for the amount of sample collected, so only the data from samples of plankton size between 50-140  $\mu m$  are presented. Lyngbya was collected only in February 2010, when it was found at Town Beach site. The collection of microphytobenthos failed several times and only samples from One Tree in October 2010 were analysed.

Statistical analysis showed that there were significant differences in  $\delta^{13}$ C and  $\delta^{15}$ N between mangroves leaves and POM, with effects of site and date (Table 12). However most of the

differences were related to the enriched value of  $\delta^{13}C$  in POM at One Tree in February 2010 and the enriched value of  $\delta^{15}N$  of mangrove leaves at One Tree in November 2009 (Figure 38).

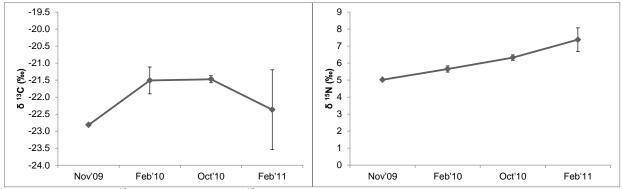
**Table 12:** Three-factor ANOVA comparing  $\delta^{13}C$  and  $\delta^{15}N$  between mangrove leaves and POM at One Tree and Town Beach in November 2009, February 2010, October 2010 and February 2011.

	F	df	р
Date	5.21	6	0.001
Site	19.35	2	< 0.0001
Source	26.25	2	< 0.00001
Date*Site	3.29	6	0.011
Date*Source	3.17	6	0.013
Site*Source	0.04	2	0.963
Date*Site*Source	1.25	6	0.306



**Figure 38.** Mean  $\delta^{13}$ C  $\pm$  SE and mean  $\delta^{15}$ N  $\pm$  SE of mangrove leaves, POM, seagrass, *Lyngbya majuscula* and microphytobenthos at One Tree and Town Beach, Roebuck Bay NWA in November 2009, February 2010 and 2011 and October 2010.

There was a significant difference in seagrass  $\delta^{15}N$  (F = 130.59, df = 3, p < 0.0001) and  $\delta^{13}C$  (F = 6.30, df = 3, p < 0.04) among dates at Town Beach (Figure 38). Plankton also showed a significant differences in  $\delta^{15}N$  ( $\chi^2 = 8$ , df = 3, p = 0.05) but not in  $\delta^{13}C$  ( $\chi^2 = 4$ , df = 3, p = 0.26) among dates (Figure 39).



**Figure 39.** Mean  $\delta^{13}$ C  $\pm$  SE and mean  $\delta^{15}$ N  $\pm$  SE of plankton (50-140 $\mu$ m) collected at Roebuck Bay NWA in November 2009, February 2010 and 2011 and October 2010. Values mean  $\pm$  SE.

#### Benthic macroinvertebrates stable isotopes analysis

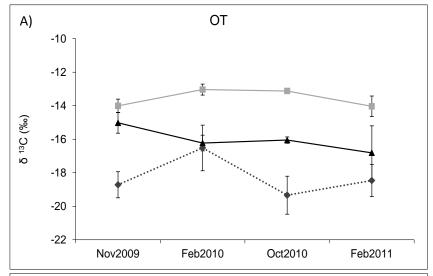
#### (Objective 2)

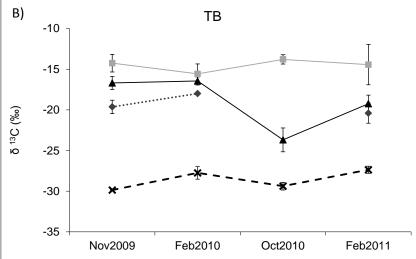
Samples of benthic macroinvertebrates were collected where possible at One Tree and Town Beach in November 2009, February 2010 and 2011 and October 2010 and grouped into feeding groups of predators, suspension feeders, deposit feeders and chemosynthetic bivalves. Chemosynthetic bivalves were collected in sufficient numbers for analysis only at Town Beach. Suspension feeders were successfully collect at Town Beach in November 2009 and February 2011 but not in in October 2010 or February 2010.

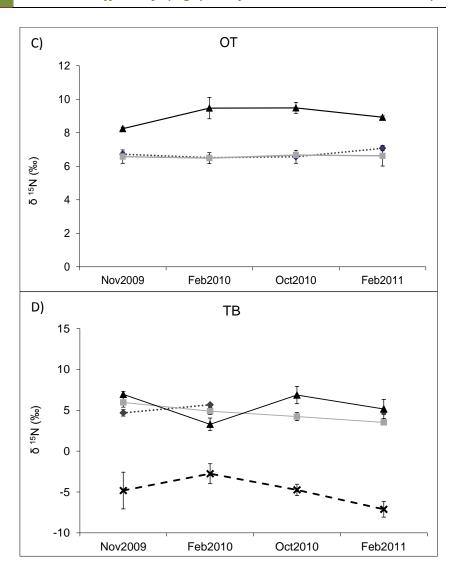
There were significant differences in  $\delta^{13}C$  and  $\delta^{15}N$  between sites, sampling dates and macroinvertebrate feeding groups found at both sites (predators and deposit feeders) and with the significant interaction factors (Table 13). Most of the differences found in  $\delta^{13}C$  were related to predators at Town Beach in October 2010, which were significantly depleted in  $^{13}C$ 

compared with all other samples (Figure 40 A)). Predators and deposit feeders at One Tree presented significant enriched values of <sup>15</sup>N compared with the same groups at Town Beach (Figure 40 C) and D)). At Town Beach chemosynthetic bivalves were significantly depleted in <sup>13</sup>C and <sup>15</sup>N (Figure 40 A) and B)) compared with all other macroinvertebrates feeding groups. At One Tree predators were significant enriched in <sup>15</sup>N compared with all other feeding groups (Figure 40 C)).

Figure 40. Mean  $\delta^{13}C \pm SE$ and mean  $\delta^{15}N$  ± SE of predators (triangles and solid line), suspension feeders (diamonds and broken line), deposit feeders (squares and solid line) and chemosynthetic bivalves (crosses broken line) collected at One Tree and Town Beach at Roebuck Bay NWA in November 2009, February 2010 and 2011 and October 2010.







**Table 13.** Three-factor ANOVA comparing  $\delta^{13}C$  and  $\delta^{15}N$  in predators and deposit feeders, between sites and among dates. (TB) Two-factor ANOVA comparing  $\delta^{13}C$  and  $\delta^{15}N$  in predators, deposit feeders and chemosynthetic bivalves at Town Beach. (OT) and the two-factor ANOVA comparing  $\delta^{13}C$  and  $\delta^{15}N$  in predators, deposit feeders and suspension feeders at One Tree. Dates are November 2009, February 2010, October 2010 and February 2011. Sites were One Tree and Town Beach.

		F	df	p
	date	3.77	6	0.002
	Site	73.40	2	< 0.00001
	Feeding guild <sup>a</sup>	69.73	2	< 0.00001
	date*Site	5.93	6	< 0.0001
	date*Feeding guild <sup>a</sup>	4.53	6	< 0.001
	Site*Feeding guild <sup>a</sup>	6.87	2	0.002
	date*Site*Feeding guild <sup>a</sup>	5.34	6	<0.0001
	date	2.73	6	0.021
ТВ	Feeding guild <sup>b</sup>	70.76	4	< 0.00001
	date*Feeding guild <sup>b</sup>	3.02	12	0.003
	date	1.64	6	0.143
ОТ	Feeding guild <sup>c</sup>	71.28	4	< 0.00001
	date*Feeding guild <sup>c</sup>	1.89	12	0.043

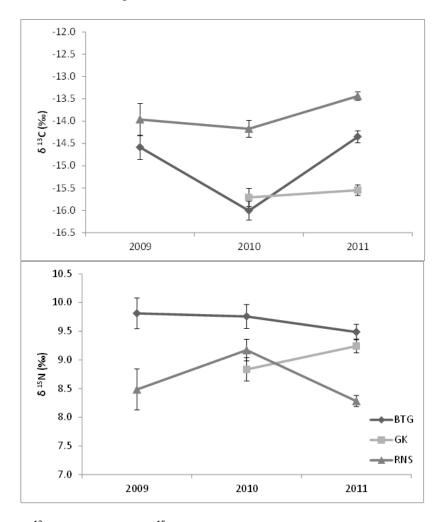
<sup>&</sup>lt;sup>a</sup> predators and deposit feeders. <sup>b</sup> predators, deposit feeders and chemosynthetic bivalves. <sup>c</sup> predators, deposit feeders and suspension feeders.

## Shorebirds stable isotopes analysis

#### (Objective 2)

Shorebirds for collection of blood samples for stable isotope analysis were captured in the northern beaches of Roebuck Bay, mostly between Dampier Creek and One Tree. Samples of Bar-tailed Godwits (*Limosa lapponica*) and Red-necked Stints (*Calidris ruficollis*) were taken in November 2009, October 2010 and February 2011 and samples of Great Knots (*Calidris tenuirostris*) were taken in October 2010 and February 2011.

There were no significant differences in  $\delta^{13}$ C (F=2.02 df = 1, p=0.16) between Bar-tailed Godwits (godwits from here on) and Red-necked Stints (stints from here on) in 2009, but there were significant between-species differences in  $\delta^{15}$ N (F=15.62, df = 1, p<0.001) for the same year. There were also significant between-species differences in  $\delta^{13}$ C and  $\delta^{15}$ N between years (2010 and 2011) (F=20.1 df = 2, p<0.00001) for godwits, stints and Great Knot (knots from here on) (F=46.3 df = 4, p<0.00001), with a significant interaction factor, specie x year (F=13.6 df = 4, p<0.00001, Figure 41). The  $\delta^{15}$ N signature of Godwits was significantly enriched compared with stints and knots (Figure 41). This species also had the most significant between-year differences in  $\delta^{13}$ C (Figure 41). The three species had  $\delta^{13}$ C minimums in October 2010 (Figure 41).



**Figure 41.** Mean  $\delta^{13}$ C ± SE and mean  $\delta^{15}$ N ± SE of Bar-tailed Godwits (diamonds), Red-necked Stints (triangles) and Great Knots (squares) captured in the northern beaches of Roebuck Bay NWA in November 2009, October 2010 and February 2011.

Only samples of knots and godwits were obtained from the same beaches during the study in 2010 and 2011. In 2010 godwits and knots were captured at Stilt Viewing and Quarry Beach (see Figure 42) and in 2011 both species were capture at Boiler Point and Eagle Roost. Boiler Point and Stilt Viewing are the locations closest to One Tree (Figure 42).

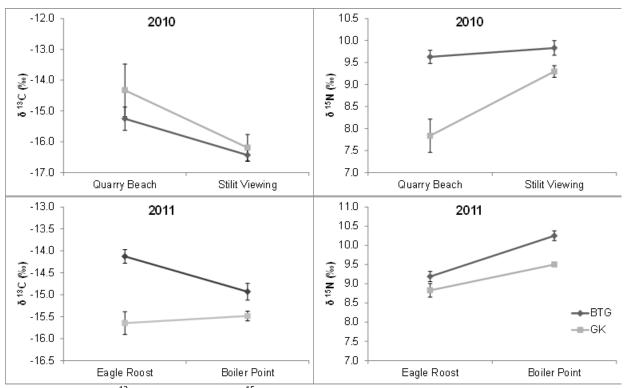


**Figure 42.** Location of some of the northern beaches of Roebuck Bay where Bar-tailed Godwits and Great Knots were captured in 2010 and 2011. NOTE: Quarry Beach and One Tree are recognised geographical locations. All other names have been created by the Australian Wader Studies Group and the Broome Bird Observatory to identify different locations and shorebird roosting beaches. They are not recognised geographical names.

In both years there was a significant effect of bird species and captured site on bird  $\delta^{13}C$  and  $\delta^{15}N$  signatures (Table 14). The  $\delta^{15}N$  of the knots captured close to One Tree were higher that the  $\delta^{15}N$  of knots captured away from One Tree (Figure 43). The same occurred with the godwits captured close to One Tree in 2011. However in 2010 the higher  $\delta^{15}N$  of the godwits captured close to One Tree was not significantly different than the  $\delta^{15}N$  of the godwits capture far from One Tree (Figure 43).

**Table 14.** Three-factor ANOVA comparing  $\delta^{13}C$  and  $\delta^{15}N$  between capture sites and species (Bar-tailed Godwits and Great knots) in 2010 and 2011. 2010 capture sites were Stilt Viewing and Quarry Beach. 2011 capture sites were Boiler Point and Eagle Roost.

		F	df	р
	Species	10.435	2	<0.001
2010	capture site	9.916	2	< 0.001
	Species*capture site	3.076	2	0.06
	Species	42.84	2	<0.000001
2011	capture site	19.15	2	< 0.000001
	Species*capture site	3.17	2	0.05

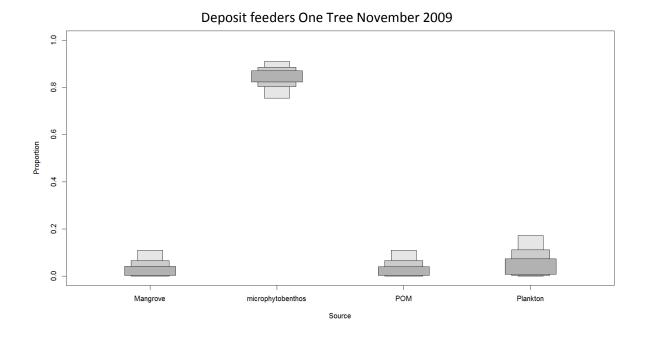


**Figure 43.** Mean  $\delta^{13}$ C  $\pm$  SE and mean  $\delta^{15}$ N  $\pm$  SE of Bar-tailed Godwits (diamonds) and Great Knots (squares) captured on the northern beaches of Roebuck Bay NWA in October 2010 and February 2011.

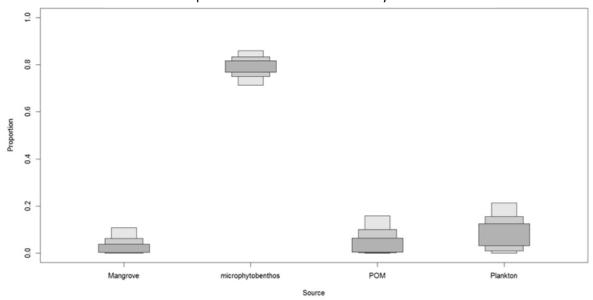
## Diet of benthic invertebrates and shorebirds using stable isotopes

The mixing models for stable isotopic analyses in R (SIAR) provided an estimate diet composition. As it is necessary to include all the potential carbon sources to carry out the mixing models (Phillips, 2012), the  $\delta^{13}$ C and  $\delta^{15}$ N values for microphytobenthos from One Tree in October 2010 were used for this site in November 2009 and February 2011. Also the  $\delta^{13}$ C and  $\delta^{15}$ N signatures of diatoms of Dampier Flats from Compton et al., (2008) were used as a source for Town Beach in November 2009 and October 2010.

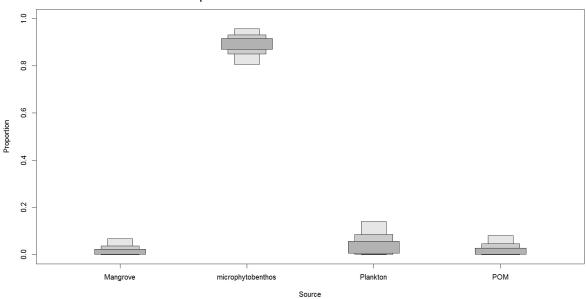
The mixing models for stable isotopic analyses in SIAR showed that the main source for deposit feeders in One Tree was the microphytobenthos (Figure 44).

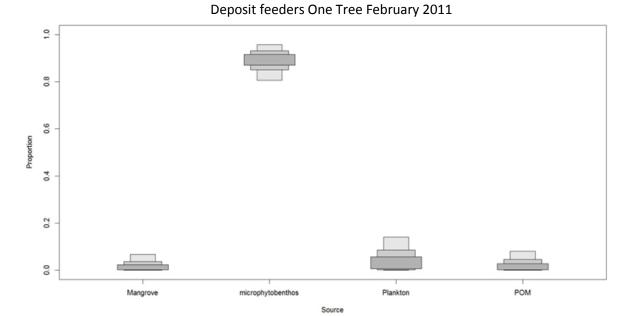


### Deposit feeders One Tree February 2010



### Deposit feeders One Tree October 2010

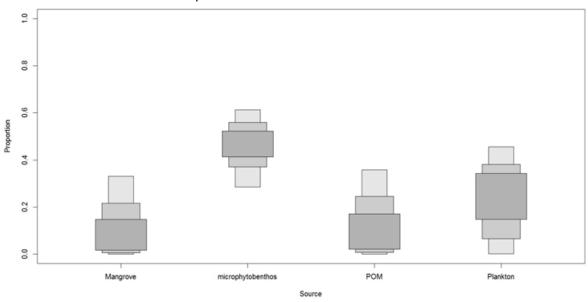




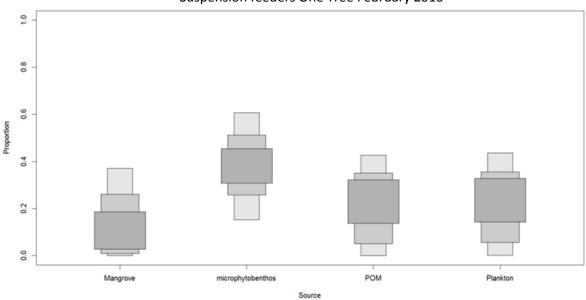
# **Figure 44.** Boxplots showing the relative contributions of potential carbon sources to the diet of deposit feeders sampled from One Tree, Roebuck Bay NWA in November 2009, February 2010, October 2009 and February 2011 from the mixing model SIAR. Credibility intervals of 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

The mixing models for stable isotopic analyses in SIAR showed that the main sources for suspension feeders in One Tree was also the microphytobenthos followed by plankton (Figure 45). However in February 2010 and October 2010 there was also an important contribution from the particulate organic matter (POM) (Figure 45).

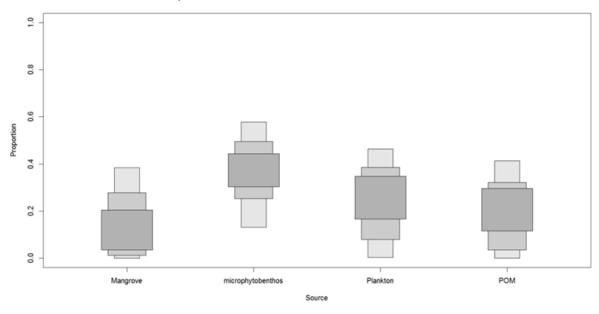
### Suspension feeders One Tree November 2009



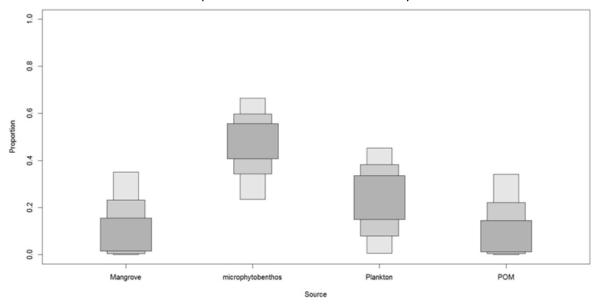
### Suspension feeders One Tree February 2010



### Suspension feeders One Tree October 2010

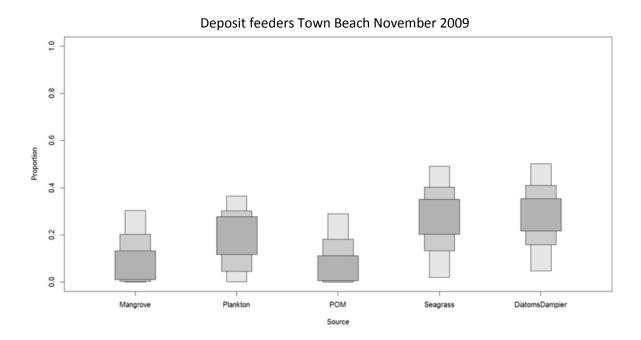


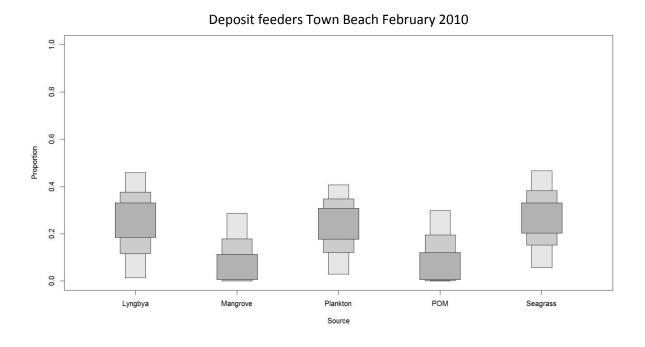
### Suspension feeders One Tree February 2011

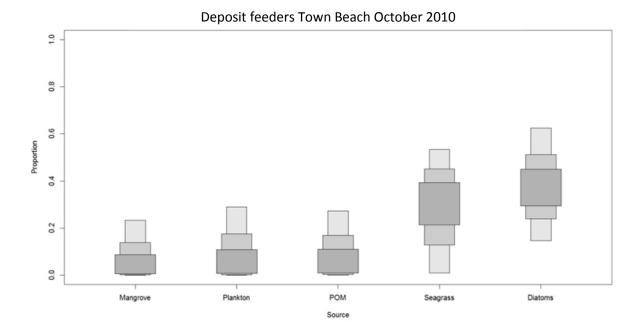


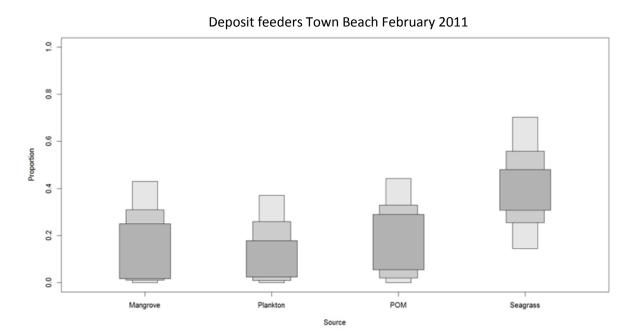
**Figure 45.** Boxplots showing the relative contributions of potential carbon sources to the diet of suspension feeders sampled from One Tree, Roebuck Bay NWA in November 2009, February 2010, October 2009 and February 2011 from the mixing model SIAR. Credibility intervals of 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

The mixing models for stable isotopic analyses in SIAR showed that the main carbon sources for deposit feeders in Town Beach were microphytobenthos and seagrass (Figure 46). However, the information provided by the SIAR for February 2010 dietary analysis was incomplete, because diatoms were not included in the analysis. SIAR indicated that in February 2010 *Lyngbya majuscula* provided an important contribution to the diet of deposit feeders (Figure 46).





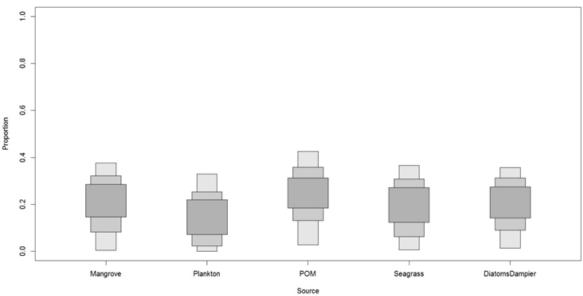


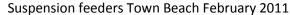


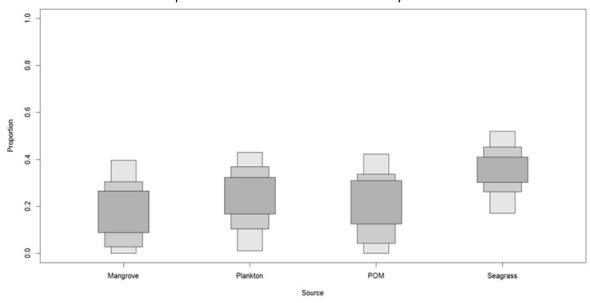
**Figure 46.** Boxplots showing the relative contributions of potential carbon sources to the diet of deposit feeders sampled from Town Beach, Roebuck Bay NWA in November 2009, February 2010, October 2009 and February 2011 from the mixing model SIAR. Credibility intervals of 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

For suspension feeders only stable isotope data from November 2009 and February 2011 were available. SIAR models showed that particulate organic matter was the main contributor to suspension feeders in Town Beach in November 2009, although with high contributions of mangroves, seagrass and diatoms (Figure 47). In February 2011 the main contributor to the diet of suspension feeders' in Town Beach was seagrass (Figure 47).

### Suspension feeders Town Beach November 2009



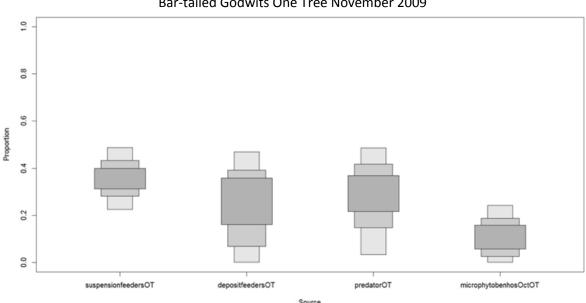




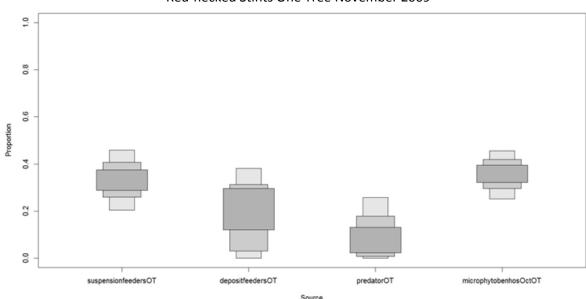
**Figure 47.** Boxplots showing the relative contributions of potential carbon sources to the diet of suspension feeders from Town Beach, Roebuck Bay NWA in November 2009 and February 2011 from the mixing model SIAR. Credibility intervals of 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

Because there was a significant effect of capture site on the  $\delta^{13}C$  and  $\delta^{15}N$  signatures of blood from shorebird, only the data from birds captured close to One Tree or from Town Beach were used to assess their diet using Bayesian multiple source isotope mixing model in R (SIAR).

For birds captured in November 2009 close to One Tree, mixing models for stable isotopic analyses (SIAR) showed that godwits assimilated suspension feeders, followed by predators as the main components of their diet (Figure 48). SIAR models indicated that microphytobenthos, with a high contribution of suspension feeders were the main components of the diets of stints (Figure 48).



Bar-tailed Godwits One Tree November 2009



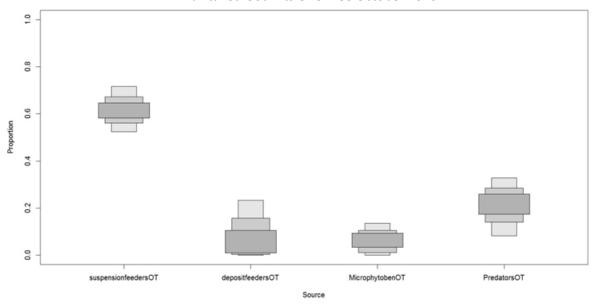
### Red-necked Stints One Tree November 2009

**Figure 48.** Boxplots showing the relative contributions of potential carbon sources to the diet of Bartailed Godwits and Red-necked Stints captured close to One Tree, Roebuck Bay NWA in November 2009 from the mixing model SIAR. Credibility intervals 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

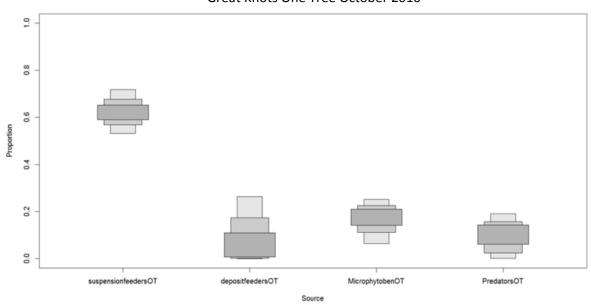
Mixing models indicated that in October 2010 the main carbon sources in the diets of Bartailed Godwits and Great Knots from One Tree were suspension feeders (Figure 49). In the case of Red-necked Stints suspension feeders also play a major role, with a high contribution of microphytobenthos (Figure 49). At One Tree in February 2010 mixing models indicated that godwits assimilated carbon primarily from deposit feeders, but with a contribution from suspension feeders and finally macroinvertebrate predators, whilst knots presented the opposite, with the majority of their carbon assimilated from suspension feeders as main, followed by deposit feeders and predators (Figure 50).

Only stints were captured in the area of Town Beach in February 2011. Bayesian mixing models (SIAR) showed that the main contribution to the diet of stints was suspension feeders followed by a high proportion of microphytobenthos (utilising diatoms, data from Compton et al. 2008) (Figure 51).

### Bar-tailed Godwits One Tree October 2010



### Great Knots One Tree October 2010



# Red-necked Stints One Tree October 2010

# **Figure 49.** Boxplots showing the relative contributions of potential carbon sources to the diet of Bartailed Godwits, Great Knots and Red-necked Stints captured close to One Tree, Roebuck Bay NWA in October 2010 from the mixing model SIAR. Credibility intervals of 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

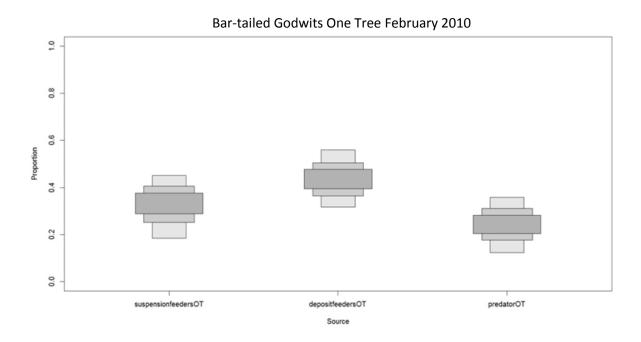
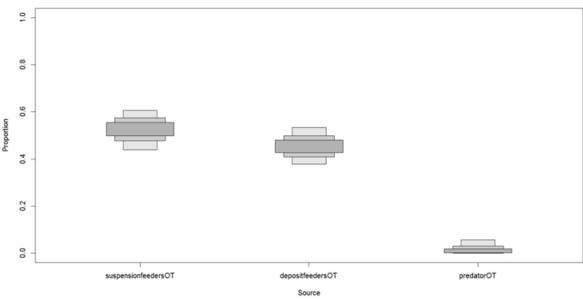


Figure 50. Boxplots showing the relative contributions of potential carbon sources to the diet of Bartailed Godwits and Great Knots captured close to One Tree, Roebuck Bay NWA in February 2011 from the mixing model SIAR. Credibility intervals 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.



Great Knots One Tree February 2011

Red-necked Stints Town Beach February 2011 0. 0.8 9.0 0.4 0.2 0.0 suspensionfeedersTB DiatomsDampier

Figure 51. Boxplots showing the relative contributions of potential carbon sources to the diet of Rednecked Stints captured close to Town Beach, Roebuck Bay NWA in October 2010 from the mixing model SIAR. Credibility intervals of 0.95, 0.75 and 0.25 are in dark grey, light grey and white, respectively.

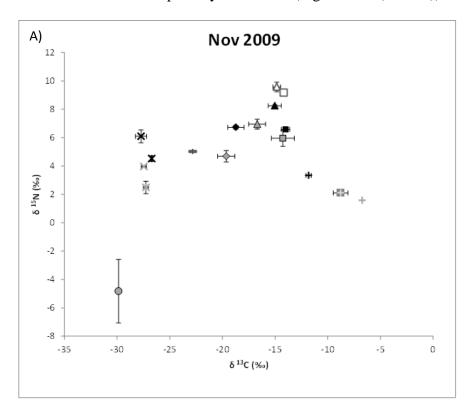
### **Food webs**

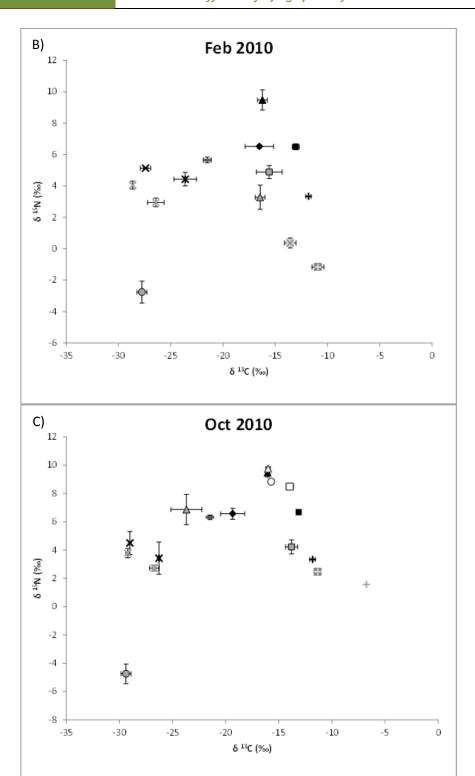
### (Objective 2)

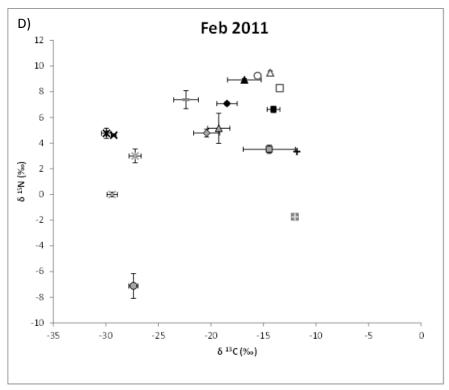
The food webs of Roebuck Bay that support shorebirds were short trophic chains with three or four trophic levels. In general, the food web of shorebirds from One Tree was nitrogen enriched compare with the food web of shorebirds from Town Beach (Figure 52 A)-D)).

At One Tree, shorebirds were in the top of the trophic chain, sharing position with macroinvertebrates predators (Figure 52 C)-D)). The food web was a simple system that had microphytobenthos as the main primary producer with only three trophic levels; primary producers, primary consumers and predators (Figure 52 A)-D)).

The food web of shorebirds from Town Beach was more complex, with several primary producers potentially playing a role as carbon sources. Shorebirds were again in the top position of the trophic chain, which had four trophic levels (Figure 52 A), C), D)), with macroinvertebrates predators lower in the trophic chain than shorebirds (Figure 52 A), C), D)) and even at or lower than some primary consumers (Figures 52 B) and D)).







**Figure 52.** Mean values of  $\delta^{13}$ C and  $\delta^{15}$ N ( $\pm$  SE) for primary producers, primary consumers and predators in One Tree (black symbols) and Town Beach (grey symbols) in Roebuck Bay, WA in A) November 2009, B) February 2010, C) October 2010 and D) February 2010. Crosses show the values for each primary producer (X mangrove leaves, XPOM,  $\blacksquare$  seagrass, + microphytobenthos and X Lyngbya) and horizontal lines — for plankton; empty symbols: shorebirds captured in the bay (ABar-tailed Godwits, ARed-necked Stint, AGreat Knot; full symbols: benthic invertebrates (APredators, AGreat Knot; full symbols: benthic invertebrates (APredators, AGreat Knot; full symbols: benthic invertebrates (APredators, APredators). Data from microphytobenthos in Town Beach is from Compton et al. 2008.



Flock of shorebirds roosting at Roebuck Bay beaches (Photo: Jose A. Masero)

# **DISCUSSION**

### **DISCUSSION**

### Lyngbya majuscula distribution and biomass

Several conditions appeared to influence the presence of *Lyngbya majuscula* in Roebuck Bay. *Lyngbya* was only present in the northern section of the Bay, which is characterised by coarser (more sandy) sediments than the rest of the Bay. Because *Lyngbya* needs high light levels for growing (Albert et al. 2005, Watkinson et al. 2005, Ahern et al. 2007, Johnstone et al. 2010, Kehoe et al. 2012, this study), the macrotidal regime of Roebuck Bay, that can result in > 9 m change in sea level, can produce greater light attenuation on the eastern intertidal areas of the Bay compared with the northern areas due to resuspension of the finer sediments (SME personal observation), and this is likely to limit or even preclude growth of *Lyngbya*. Also, the absence of a more solid substratum to attach to (there is no intertidal seagrass meadows in that part of the Bay) as well as the general instability of the finer, unconsolidated sediment that type of sediment may limit the establishment of *Lyngbya*.

The main climatological factor correlated with high *Lyngbya* biomass in Roebuck Bay was the onset of thunderstorm activity and heavy rains in December. These intermittent heavy rains, combined with long clear periods providing high radiant light in the same month, together with increasing ambient temperatures, appear to drive *Lyngbya* blooms in Roebuck Bay. The relationship with heavy rains at the start of the wet season is probably link to flushing of nutrients from the local catchment resulting in input to the system of nutrient rich runoff, as has been observed in other studies (Ahern et al. 2006). Once nutrients levels have increased in the system, *Lyngbya* only needs high ambient light and warm temperatures to stimulate growth (Albert et al. 2005, Dennison et al. 1999; Watkinson et al. 2005, Ahern et al. 2007, Johnstone et al. 2010).

The negative correlation between *Lyngbya* biomass and concentrations of ammonium and phosphorus in the sediment showed that the sediments are one of the main contributors of *Lyngbya*. The fact that sediment nutrients pool plays a major role in *Lyngbya* growth compared with nutrients in the water column has been already establish in other studies (Johnstone et al. 2007, Johnstone et al. 2010). The inverse relationship between *Lyngbya* biomass and low concentrations of ammonium and phosphorus in sediments may indicate that *Lyngbya majuscula* initially established on sediments rich in ammonium and phosphorous but subsequently depleted these nutrients from the sediment through high growth of *Lyngbya*. Supporting this, phosphorus has already been noted as one trigger of blooms of *Lyngbya* in Moreton Bay (Elmetri and Bell 2004, Ahern et al. 2008).

Lyngbya majuscula is capable of N<sub>2</sub> fixation (Jones 1990, Phlips et al. 1991, Olson et al. 1999, Lundgren et al. 2003, Joyner et al. 2008), but it can also assimilate inorganic forms of nitrogen (Ahern et al. 2007, Paerl et al. 2008). In fact, Watkinson et al. (2005) indicated that Lyngbya could use ammonium as a nitrogen source and in Florida L. majuscula and L. polychroa have shown a preference for ammonium uptake (Paerl et al. 2009). Moreover, the nitrogen fixation and the synthesis of nitrogenase by Lyngbya majuscula has been found to occur only at night while during the daylight hours nitrogenase was degraded to undetectable levels (Lundgren et al. 2003). So other forms of inorganic nitrogen must be used by Lyngbya during daylight hours. Recent studies have reported that at least some strains of Lyngbya majuscula strains are unable to fix atmospheric nitrogen (Jones et al. 2011). It is unknown if the Lyngbya majuscula found in Roebuck Bay belongs to this strains.

It has been established that bioavailable iron is known to limit *Lyngbya* blooms in other systems (Elmetri and Bell 2004, Ahern et al. 2008). However, the PLS<sub>nutrient</sub> model did not find a correlation between bioavailable iron and *Lyngbya* biomass in Roebuck Bay. This was, probably because bioavailable iron was either found at very low levels or below the detection

detection in the two seasons (wet season 2010-2011 and wet season 211-2012) when nutrient analyses were performed. However, Lyngbya biomass was relatively low in both these seasons compared with the previous wet season (2009-2010), when there was an extensive and dense *Lyngbya* bloom in Roebuck Bay. Unfortunately there are no data on nutrient concentrations in sediments or water for the season when Lyngbya was in bloom, and so the levels of bioavailable iron are unknown. Therefore it should not be ruled out that bioavailable iron plays a role in limiting *Lyngbya* blooms in Roebuck Bay.

In summary, the present study demonstrated that blooms of *Lyngbya* in Roebuck Bay are dependent on concentrated heavy rains in December, extended periods of sunny days within the same month, warm temperatures in January and sediments rich in ammonium and phosphorous.

### Nutrients, water quality and nutrient enrichment

It was hypothesised that, given the location of *Lyngbya* blooms, nutrient concentrations would be elevated in sediments and waters close to the town of Broome (stations Port of Broome and Town Beach). However, analyses have demonstrated that nutrient concentrations in water and sediment were generally higher at the stations situated in the northeast of the Bay (Fall Point and One Tree). This may be because this section of the Bay is characterised by fine sediments which tend to accumulate organic material and nutrients (Erftemeijer and Middleburg 1993, Andrieux-Loyer and Aminot 2001), whereas sediments at sites close to the town were generally coarser. Nutrient fluxes are a normal sediment—water interaction (Klump and Martens 1981), so sediment nutrient concentrations can easily affect water nutrient concentrations. Nevertheless, the lack of knowledge about the currents and circulation pattern in the Bay has made it impossible to determine the origin of the nutrients

found on the northeast section. The stations close to Broome (Town Beach and Port of Broome) however showed higher concentration of nutrients in water and sediment than the stations situated on the northern area of the Bay (Dampier Creek and Camp Site). When compared against water quality guidelines, on several occasions total nitrogen concentrations in water, considered the primary limiting element in marine systems (Howarth 1988), were in exceedance of current ANZECC/ARMCANZ (2000) default trigger values in all stations. The high concentration of nutrients in water and sediments of the Bay together with the biological information gathered in the present study support the proposition that there is significant nutrient enrichment in Roebuck Bay, as suggested by previous studies (Storey unpub. data, RBWG 2008).

The present study also draws attention to a potential eutrophication process in Roebuck Bay. The study recorded several ecological processes that typify different phases of established models of eutrophication of marine systems (Gray 1992, Cloern 2001). During the study high concentration of nutrients were recorded in water and sediments in the Bay, with a significant increase in sediment organic matter content. Subsequently there was an extensive and dense bloom of a fast growing opportunistic primary producer (toxic cyanobacteria *Lyngbya majuscula*). This in turn resulted in changes in benthic macroinvertebrate community assemblages and diversity, with for example an obvious increase in the abundance of one taxa of deposit feeder known to be tolerant of hypoxic conditions (sipunculids). Observations of shorebird feeding then noted a change in top predators foraging behaviour and diet as a result of the alteration in benthic macrofauna, and therefore trophic links were also modified.

The fact that *Lyngbya* does not present extensive and dense blooms every year in Roebuck Bay does not mean that the potential eutrophication process has stop. As has been noted in the present study and also in the literature (Kehoe et al. 2012), *Lyngbya majuscula* needs specific conditions to bloom, heavy rain and high ambient light, sediment rich in nutrients

and available iron. If all of these parameters are not present, then is likely *Lyngbya* will not bloom. It should also be noted that just because a bloom was not recorded in some years, it does not mean Lyngbya was absent. It was recorded in each year, albeit in much reduced coverage and biomass in non-bloom years. This indicates that Lyngbya is established in the Bay, and with bloom when conditions permit. Also, the fact that nutrient concentrations in the water were above the default ANZECC/ARMCANZ (2000) trigger levels at the end of the study indicates ongoing eutrophication, and not a once-off sporadic event. It is possible that shifts towards other opportunistic primary producers such as phytoplankton or macroalgae, and other effects of eutrophication, are occurring in the Bay as the increase in sediment organic matter indicates (Martin et al. 2011). So, further research is needed to fully understand the implications of elevated nutrient levels for the ecology of the system.

### Abundance and composition of benthic macroinvertebrate assemblages

Roebuck Bay represents a typical tropical intertidal habitat, with high abundances of crabs, polychaetes and bivalves (Alongi 1989, Dittman 2001). The sandy site of Town Beach, with seagrass meadows was characterised by a high diversity of macroinvertebrates represented by a wide range of taxa, such as polychaetes, amphipods, isopods, ostracods, shrimps, crabs, brittle starts and bivalves. The muddy site of One Tree on the other hand presented a much lower diversity and its representative taxa were bivalves, crabs and tusk shells.

The presence of *Lyngbya majuscula* at high densities (mean biomass > 300 g AFDM) significantly affected the composition, abundance and diversity of benthic macroinvertebrates in those parts of Roebuck Bay affected by the bloom. Conversely, lower densities of *Lyngbya* did not appear to significantly affect composition, abundance or diversity of benthic macroinvertebrates.

The effects of Lyngbya were different at different sites. For instance in February 2010 there were significant declines in macroinvertebrate diversity in areas cover by seagrass where the highest biomass of Lyngbya occurred, or in sandy areas close to the seagrass beds (Town Beach). On the other hand macroinvertebrate diversity increased in soft sediment areas far away from the Lyngbya bloom (i.e. One Tree). At Town Beach the decrease of diversity was related to the dramatic increase in the abundance of sipunculids and gastropods, taxa considered to be tolerant of hypoxic conditions (Langenbuch and Pörtner 2004; Vaquer-Sunyer and Duarte 2008). It is well documented that blooms of Lyngbya majuscula produce significant biomass of organic material (Ahern et al 2007, this study). Such a large biomass increases the oxygen requirements to metabolise the organic matter load which can lead to hypoxic or anoxic conditions, as has been observed in Moreton Bay (García and Johnstone 2006). At the Town Beach station, situated on bare sand 150 m offshore, there was a larger decrease in diversity compared to the station situated on the seagrass bed, 250 m offshore. Seagrass beds generally support greater macroinvertebrate diversity than other intertidal areas due to greater habitat complexity (Howard et al. 1989, Hyndes et al. 2003, Unsworth et al. 2007). Also, the invertebrate fauna of unvegetated areas close to seagrass beds are known to be more sensitive to anoxic conditions than the fauna of seagrass beds (Fonseca et al. 2011), which may explain the large decline in diversity at the site 150 m at Town Beach during the Lyngbya bloom in February 2010. At One Tree the increased of diversity observed in February 2010 reflected an increase in the number and abundance of several families, particularly polychaeta which tended to be deposit feeders and predators. It is possible that the particulate organic matter resulting from the Lyngbya bloom in February 2010 reached One Tree in a more dilute form compared with Town Beach and the availability of this organic matter was enough to increase the number of species and abundance of different deposit feeders, followed by an increase in predators.

Some of the variability in benthic macroinvertebrates abundances observed in Roebuck Bay, particularly outside the February 2010 bloom, could also be related to environmental conditions not linked with Lyngbya blooms. For example, Unsworth et al. (2010) found that carideans on tropical seagrass meadows showed changes in abundance related to the tidal, diel and moon cycles. Although all of our sampling for the current study was carried out at spring low tides during daylight hours, samples were collected at different periods during the day (sunrise, midday or afternoon) and in different seasons. Another factor that could also affect benthic macroinvertebrate abundance was seagrass biomass. Seagrass biomass has been related to changes in macrobenthos abundance in other tropical intertidal areas of Australia (Klumpp and Kwak 2005). In the first months of 2012, the seagrass meadows at Town Beach were rated as rated as poor, with lower coverage than in previous years (Mckenzie et al. 2006-2013). This may have contributed to the low abundances of several macroinvertebrate taxa observed at the end of the study period. Nevertheless the low abundance, high diversity and high variability in abundance of macroinvertebrates, together with the general lack of knowledge on population dynamics (Dittmann 2001) or seasonality in recruitment patterns (de Goeij et al. 2003), makes the interpretation of temporal patterns in benthic macroinvertebrates in tropical intertidal flats elusive.

### **Shorebird foraging behaviour**

Direct observation of foraging Bar-tailed Godwits showed that sentinel crabs were the most common prey at Town Beach and at One Tree through most of the study, in accordance with other studies (Zharikov and Skilleter 2002). However, in February 2010, when *Lyngbya* was present, a shift in diet occurred towards sipunculids at Town Beach, where they were the most common prey, peaking at more than 3000 individuals per square meter. As in other

situations, this change in behaviour suggests that godwits utilise an opportunistic foraging strategy (Davis and Smith 2001, Skagen 2006) to exploit the available, high density and low mobility sipunculids. This shift in prey utilisation, however, did not appear to have any implications for biomass acquisition for this long distance migratory bird with high energetic demands (Meta et al. 2005). In fact, analysis indicated there was no significant difference in the intake rates obtained at Town Beach in February 2010 compared with estimates obtained from the same site in February 2011. However, the highest intake rates of the whole study were obtained at Town Beach in November 2009, before the *Lyngbya* bloom of February 2010, reflecting to the high number of prey captured per minute by the Bar-tailed godwits.

In general, there was no significant difference in the biomass obtain per minute between sites, which indicates that both locations provided similar quality feeding grounds, although offering different prey items. However, other factors such as differences in predation risk (Cresswell and Whitfield 2008) or disturbance (e.g. human interference) (Yasué 2005) can affect site quality.

### Stable isotopes and food webs

Stable isotope analysis indicated that microphytobenthos (MPB) plays a crucial role in providing energy (carbon) to the food web that supports shorebirds in Roebuck Bay. This role is either by direct ingestion by some species of birds such as Red-necked Stints (Kuwae et al. 2012, this study), or as a carbon source supporting lower trophic levels, especially in soft muddy areas. Previous studies have reported that mangroves provide a limited carbon input to food webs of tropical intertidal habitats (Heithaus et al. 2011), and that MPB plays a major role as a carbon source for macrobenthos trophic levels (Bouillon et al. 2002, Bouillon 2008). In soft sediment areas of Roebuck Bay, even suspension feeders had a strong signal

indicating MPB as a major carbon source, a fact that has also been described in other systems (Kang et al. 1999, Kang et al. 2003). In estuaries, resuspension of microphytobenthos is an important phenomenon especially in areas of fine sediments (Ubertini et al. 2012), and is affected by winds and tides (de Jonge and van Beusekom 1995) and can often exceed the biomass of phytoplankton in tidal flat ecosystems (de Jonge and van Beusekom 1992). In addition, there is generally higher MPB biomass in muddy rather than sandy sediments (Cartaxana et al. 2006). Therefore, since Roebuck Bay is a macrotidal system, with large areas of fine sediment, it is expected there would be high rates of microphytobenthos resuspension, which may explain the inclusion of microphytobenthos as a major energy source for suspension feeders particularly at diet in One Tree.

Seagrass was also an important primary producer supporting the food web of shorebirds in the western part of the Bay. However carbon sources were more variable in that section of the Bay with, for example Lyngbya appearing as a main carbon source for deposit feeders in February 2010. Seagrass exhibited presented a seasonal pattern in  $\delta^{15}N$ , with maximum  $\delta^{15}N$  at the end of the dry season and minimum values in the wet season. It has been hypothesised that the seasonal pattern of  $\delta^{15}N$  in seagrass in Florida is related to higher requirements for nitrogen during the summer growing phase, with greater  $\delta^{15}N$  in summer and lower  $\delta^{15}N$  in winter (Fourqurean et al. 2005). Seagrass coverage in Roebuck Bay appeared to be higher at the end of the dry season and lower at the end of the wet season (Mckenzie et al. 2006-2013), which is in accordance with the observed  $\delta^{15}N$  pattern proposed by Fourqurean et al. (2005).

It is likely that the role play as primary producers by seagrass and Lyngbya, when it is present, in the food webs near Town Beach is greater than this study suggests. But the lack of data on grazers makes it difficult to prove this assumption. Nevertheless, benthic macroinvertebrate predators at Town Beach showed a similar  $\delta^{15}N$  signal as seagrass, which may indicate predation on seagrass grazers. In fact, macroinvertebrate predators were  $\delta^{15}N$ 

enriched approximately twice the fractionation factor ( $2.45 \pm 2.5$ , Vander Zanden and Rasmussen 2001) compared with seagrass values.

The higher concentrations of nitrogen in sediments and water at One Tree can be tracked through the food web. Primary consumers and shorebirds taken close to One Tree had enriched  $\delta^{15}N$  compared with macroinvertebrates collected from Town Beach and shorebirds captured some distance from One Tree. The fact that the  $\delta^{15}N$  signature of shorebirds corresponded with their capture location, and therefore with their feeding grounds, suggests that shorebirds in Roebuck Bay had a high fidelity to their feeding sites, roosting close to their feeding grounds. This was previously concluded by Rogers (2006).

Direct observation of Bar-tailed Godwits feeding at One Tree recorded the deposit feeding, fiddler crab, as their main prey item. On the other hand, the SIAR model indicated that suspension feeders, mainly bivalves were the main source for Bar-tailed Godwits at One Tree. This difference between approaches could indicate that the  $\delta^{13}$ C and  $\delta^{15}$ N of the blood of Bar-tailed Godwits reflects carbon sources assimilated as opposed to items ingested but not contributing energy (Phillips 2012). The suspension feeding bivalves at One Tree, mainly *Siliqua pulchella* and Tellinidae have thinner shells than crabs, which would produce less ballast (indigestible) material. Ingested ballast material could constrain digestion (Zwarts and Blomert 1993, van Gils et al. 2005) and reduce assimilation efficiency (Speakman 1987). As a result, ingested crabs could be underrepresented in the carbon signature of the blood of godwits due to lower assimilation efficiencies.

The food webs that support shorebirds in Roebuck Bay are short food chains since they only have between three to four trophic levels. In other systems like tropical rivers with highly productive short food webs, predators also occupy lower trophic levels (Layman et al. 2005). A system that presents a high diversity of primary consumers allows predators to prey on a

wide range of primary consumers (Layman et al. 2005), which appears to be the case for Roebuck Bay. However, food web structure is a key factor in ecosystem response to perturbations and simple food webs with lower complexity are not robust against perturbations (Dunne et al. 2002). It follows that the high reliance of the One Tree food web on MPB may increase this food web's sensitivity to any potential perturbation affecting the MPB.



Roebuck Bay from One Tree (Photos: Tom de Silva)

# **CONCLUSIONS**

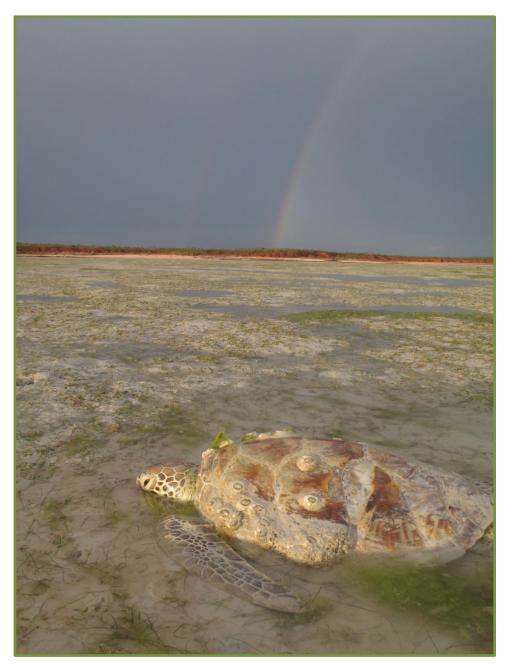
### **CONCLUSIONS**

- Blooms of *Lyngbya* in Roebuck Bay are dependent on concentrated heavy rains in December, extended periods of sunny days within the same month, warm temperatures in January and sediments rich in ammonium and phosphorous.
- Lyngbya majuscula in Roebuck Bay is found in the northern and north-westerly intertidal areas of the Bay. Two hot spots of Lyngbya biomass were identify, one between Port of Broom and Town Beach, and the other between Dampier Creek and the Broome Bird Observatory (Fall Point).
- Nutrient (N and P) concentrations in the coastal waters of Roebuck Bay are above the trigger values indicated in the ANZECC/ARMCANZ (2000) water quality guidelines.
- High levels of nutrients (N and P) together with the opportunistic blooms of cyanobacteria *Lyngbya majuscula* are indicative of nutrient enrichment and potentially eutrophication.
- Blooms of *Lyngbya majuscula* significantly affected and modified the benthic invertebrate community of Roebuck Bay.
- The induced changes in the benthic invertebrate community of Roebuck Bay have had a cascade effect on the foraging behaviour of at least one species of long distance migratory shorebird, the Bar-tailed Godwit (*Limosa lapponica*), whose diet was modified in presence of high density *Lyngbya* blooms.
- The induced dietary change of Bar-tailed Godwits due to the modification of the benthic invertebrate community did not decrease their biomass intake per minute.
- Direct observations of Bar-tailed Godwits feeding behaviour showed that the main prey for this shorebird species in Roebuck Bay were the sentinel crabs (*Macrophthalmus sp.*).
- The sampling sites with fine sediment grain size on the eastern coast of the Bay had higher sediment and water concentrations of nutrients (N and P) than other areas of the Bay,

including Town Beach. This finding aligns with the higher adsorption capacity of fine sediments for nutrients. The sampling sites with sandy sediments presented lower concentrations of nutrients.

- Within the sandy areas of the Bay, the sampling sites close to Broome presented higher water and sediment nutrient concentrations than the sites between Dampier Creek and One Tree.
- The nutrient enriched sediments of the Bay are related with food webs enriched in nitrogen. Therefore, the primary consumers and final predators (shorebirds) that were captured close to the enriched sediment site (One Tree) presented enriched levels of the isotope <sup>15</sup>N.
- Shorebirds presented high fidelity towards their feeding grounds in Roebuck Bay, as was pointed out by the stable isotope signatures. The birds that were captured on the eastern beaches of the Bay presented significantly higher levels of the stable isotope <sup>15</sup>N compared with the birds captured on the western beaches of the Bay. This finding indicates that the former were feeding in the easterly enriched mudflats while the latter fed on the north-westerly mudflats of the Bay.
- Stable isotope analysis indicated that in the eastern area of the Bay, the main prey for Bar-tailed Godwits were suspension feeders followed by predators then deposit feeders in the dry season, with deposit feeders being a more predominant source than suspension feeders in the wet season. Great Knots (*Calidris tenuirostris*) had suspension feeders as a main source in both seasons. Red-necked Stints (*Calidris ruficollis*) had as main source microphytobenthos followed by suspension feeders and lastly, predators. On the north-westerly beaches of the Bay, only Red-necked Stints were captured and their main prey were suspension feeders followed by microphytobenthos.

- The food webs of Roebuck Bay that supported shorebirds were short trophic chains with three or four trophic levels. The One Tree food web was a simple system that had only three trophic levels, with shorebirds at the top of the trophic chain. The Town Beach food web had four trophic levels, with shorebirds again in the top position of the trophic chain.
- The food webs that supported the shorebird community of One Tree consist of microphytobenthos followed by plankton as primary producers while the respective breakdown for the Town Beach area was seagrass followed by microphytobenthos then particulate organic matter.



Turtle at low tide on seagrass beds, Roebuck Bay. (Photo: Tom de Silva)

## MANAGEMENT RECOMENDATIONS

### MANAGEMENT RECOMMENDATIONS

The current study identified several elements that should be considered in order to address the development of blooms of *Lyngbya majuscula* in Roebuck Bay. It is not possible to control the bloom once it has developed, the appropriate approach is to implement management actions to prevent bloom formation in the first instance. These actions should target those mechanism and processes that are under human control. Data from this and other studies indicate that nutrient levels in the Bay are elevated above water quality guidelines. Eutrophication is an acknowledged driving force for algal blooms, and therefore the main recommendation is to avoid eutrophication of the system, and thereby make nutrients limiting; this clearly means to *reduce the input of nutrients into Roebuck Bay*. However, the lack of knowledge about nutrient sources and the hydrodynamics of Roebuck Bay make this task difficult. Further work is required to identify the source(s) of nutrients entering the Bay, and then implement management actions to reduce nutrient loads to the system, and also to understand the hydrodynamics of the Bay, especially circulation patterns, tidal currents and extent of flushing.

### Other actions to consider are:

• Public awareness: In areas where toxic cyanobacterial blooms occur, it is important that information is provided by local authorities to the general public to raise awareness. At different stages of the bloom, Lyngbya can be toxic, resulting in adverse reactions in humans when touched. This may be in the form of lesions, eyes and skin irritation and breathing problems. The public need to be made aware of these issues when a bloom is active. The written press, radio, television and internet are all valuable means to inform the public. At the same time warning signage should be posted along the waters edge in the vicinity of areas affected by a bloom. At an early

stage, the health officer and local medical personnel need to be provided with information on health issues associated with the bloom, including how to diagnose and treat affected individuals. Important information points that could be provided for the general public when *Lyngbya* blooms occurs are:

- The risks of bathing or sporting activities in contact or in close proximity to Lyngbya during a bloom event;
- Health issues related with bathers or people walking along shores of areas affected by blooms or along the shore where algae has accumulated and dried;
- The health risk connected to collecting and eating fish and shellfish during a bloom event.
- Inform medical personnel from community services: Medical personnel should be informed of the presence of an active bloom. Also they should be aware of symptoms of adverse reactions.

### **Gap knowledge recommendations**

There are several knowledge gaps that this study was unable to cover but that require attention in order to provide a more holistic view of the biotic and abiotic process that affects the environment of the Bay and that are link to nutrient enrichment and blooms of *Lyngbya majuscula*.

• *Identification of nutrient sources*. To prevent eutrophication of the system, and reduce the risk of ongoing Lyngbya blooms, it is important to identify the major sources of nutrients entering the Bay. Once identified, it will be possible to manage the sources. Identification of sources will require studies of the catchment feeding the Bay. Knowledge of industrial activities, discharge practices and locations, as well as agricultural practices (fertilizer

contribution/plant use and localization of crops, stock yards, grazing areas) is necessary in order to plan and implement actions aiming at limiting further nutrient inputs to the system. The identification of sewage discharge points, agricultural practices, the nature of the soil, the vegetation, and the interaction between the soil and the water can be of great help in knowing which areas should be targeted. The study by PhD researcher, Gayan Lakendra Gunaratne under the supervision of Assoc. Prof. Ryan Vogwill, Assoc. Prof. Matt Hipsey and Assoc. Prof. Ryan Lowe "The effects of altered hydrological regimes on water quality and nutrient delivery to a sub-tropical coastal transitional wetland" and funded by UWA and DEC will help address this objective.

- Knowledge of the hydrodynamics of the water body. Currently there is limited understanding of the circulation patterns, tidal pathways, run-off patterns and extent of flushing of the Bay. Such knowledge is required, particularly to understand how nutrients are transported into and around the Bay, and will allow determination of the processes through which the water is enriched with nutrients, and how these nutrients are transported. The study by PhD researcher, Gayan Lakendra Gunaratne (UWA) under the supervision of Assoc. Prof. Ryan Vogwill, Assoc. Prof. Matt Hipsey and Assoc. Prof. Ryan Lowe "The effects of altered hydrological regimes on water quality and nutrient delivery to a sub-tropical coastal transitional wetland" and funded by UWA and DEC will help address this objective.
- Determine the effects of Lyngbya blooms on the ecological health of seagrass meadows of Roebuck Bay. The current study looked at how Lyngbya blooms impacted abundance, diversity and composition of sediment-dwelling macroinvertebrates in areas affected by blooms. The study observed blooms on seagrass beds, but the effects of blooms on the ecological health of seagrass beds was not investigated. The importance of seagrass beds as nursery areas for fish and prawns is well acknowledged. It is important to investigate how Lyngbya blooms affect seagrass beds by modifying sediment and water conditions as well as

direct impacts on the fauna of seagrass beds and the seagrass beds themselves by smothering by *Lyngbya*.

- Assess how Lyngbya blooms modify macrobenthic communities and their capacity to recover by modifying benthic sediment conditions. The current study investigated the impacts of Lyngbya blooms on benthic fauna, but was not able to investigate the mechanisms through which Lyngbya cause impacts. It is recommended to investigate how Lyngbya blooms modify benthic sediment abiotic conditions (i.e. oxygen levels, pH, temperature), and how these conditions change following a bloom and how the benthic macroinvertebrate community recovers after a Lyngbya bloom. The Honours project of Thomas de Silva under the supervision of Dr. Mike Van Keulen, Dr. Navid Moheimani and Dr. Sora M. Estrella would cover this objective.
- Identify the potential effects that Lyngbya blooms have on the vertebrate seagrass foragers' community (dugongs and turtles). As seagrass grazers, turtles and dugongs may ingest Lyngbya majuscula growing on seagrass beds. Previous studies have linked the presence of Lyngbya with changes in marine turtle's blood biochemistry (Arthur et al. 2008). Also the presence of a Lyngbya tumour promoting compound in turtle tissue was related with the existence of skin tumours (Arthur et al. 2008). Therefore it is important to asses and monitor the health condition of Roebuck Bay dugongs and turtles when Lyngbya blooms are active and when Lyngbya is not present.
- Evaluate the impact that successive Lyngbya blooms could have on fisheries and oyster culture in Roebuck Bay. In other regions affected by Lyngbya blooms, such as Moreton Bay, fish catches were significantly lower in years with Lyngbya blooms than in years without blooms (Pittman and Pittman 2005). Also, the presence of cyanobacteria contamination in the diet of bay scallops had an effect on the survival and growth rates of the cultivated scallops

(Meseck et al. 2007). At the same time it has been proved that bivalves such as oysters, mussels, clam and scallops are able to accumulate cyanobacteria toxins that may lead later to seafood poisoning (Ibeling and Chrous 2007).

• Assess if Lyngbya majuscula toxins are transferred through the food web to higher trophic levels. Evaluate if bioaccumulation and biomagnification of Lyngbya toxins occurs at distinct trophic levels within the food web. Cyanobacteria toxins have been reported to be transferred though the food web to higher trophic levels in fresh and sea water systems (Ibeling et al. 2005, Lehman et al. 2010). However, it is not known if this process is taking place in Roebuck Bay.

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