Effects of *Lyngbya majuscula* on the diversity and abundance of benthic macroinvertebrates during an in situ simulated bloom.

Thomas de Silva

Honours Thesis (Marine Science)

Supervisors:

Dr. Navid Moheimani

Dr. Mike Van Keulen

Dr. Michael Borowitzka

Dr. Sora Marin-Estrella

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Abstract

Harmful algal blooms have increased in coastal and estuarine waters in recent years. Over the past 5 years, harmful blooms of the toxic cyanobacterium *Lyngbya majuscula* have increased in frequency in Broome, Western Australia, with blooms in Roebuck Bay hypothesised to be linked to increased nutrient inputs from anthropogenic sources. An in situ field experiment was conducted on Simpson's Beach (Roebuck Bay), Western Australia, to determine the effects of *Lyngbya majuscula* on the abundance and species richness of macroinvertebrate in the area. The formation of a natural bloom did not occur during the period of this study. A bloom was then simulated from data previously collected in the area by the gradual addition of *Lyngbya majuscula* to enclosed quadrats. Samples were taken every six days over a two month period (53 days) during low tide and included macroinvertebrate, sediment grain size, dissolved oxygen, pH, temperature and nutrients. The most affected taxa of macroinvertebrates due to the addition of high amounts of *Lyngbya* were the gastropods, followed by bivalves and polychaetes, which all decreased in abundance. Sediments changed very little over the period of the study but overall the inclusion of *Lyngbya* resulted in an increase in the amount of coarse sand found in the quadrats. Dissolved oxygen increased in time in the presence of *Lyngbya majuscula* and pH was generally lower under the same treatment conditions but results were not conclusive. No significant differences were found between the nitrate of quadrats with *Lyngbia majuscula* biomass and control. However, phosphates decreased significantly after higher amounts of *Lyngbya majuscula* were obtained in the impact treatment. The experiment shows the effects of *Lyngbya majuscula* on macroinvertebrates as an overall reduction in abundance.

Introduction

Increased nutrient loads have allowed for the introduction and increased distribution and occurrence of harmful algal blooms in estuaries and coastal waters across the world (Dennison *et al.* 1999; Ahern *et al.* 2007; Ahern *et al.* 2008; O' Neil *et al.* 2012). Harmful algal blooms are those which cause damage to ecosystems, human health, fishery resources, recreational activities and financial income such as tourism through the smothering of habitats such as mangroves, corals and seagrass (Ahern *et al.* 2008; Estrella 2013). Furthermore some of these harmful species are toxic (Codd *et al.* 1999; Osborne *et al.* 2008; Taylor *et al.* 2014). One such harmful species that is increasing its distribution and occurrence is the benthic nitrogen-fixing toxic marine cyanobacterium *Lyngbya majuscula* (Family Oscillatoriaceae), otherwise known as fireweed or maiden's hair (Dennison and Abal 1999; Ahern *et al.* 2008).

Lyngbya can grow rapidly when in a suitable habitat under favourable growth conditions, such as; optimum temperature and solar radiation, and bioavailable nutrients (nitrogen, phosphorous and iron) (Paerl *et al.* 1987; Postgate 1987; Ahern *et al.* 2008; O' Neil *et al.* 2012). *L. majuscula* can form many olive-grey unbranched filamentous strands (Figure 1). *L. majuscula* can attach onto rock outcrops, mangroves, corals, seagrass, buoys and macroalgae (Dennison *et al.* 1999; Albert *et al.* 2005; Paul *et al.* 2005; Garcia and Johnstone 2006). Once attached, *L. majuscula* can smother these substrates by forming benthic mats at depths of up to 30 m (Dennison *et al.* 1999; Albert *et al.* 2005; Ahern *et al.* 2007). These mats limit light penetration, take up available nutrients and deplete oxygen during decomposition resulting in detrimental conditions for the entire ecosystem, particularly autotrophic producers (Dennison *et al.* 1999; Stielow and Ballantine 2003; Paul *et al.* 2005; Garcia and Johnstone 2006).

Figure 1. *Lyngbya majuscula* found at Roebuck Bay, Broome, Western Australia and taken from Blois, Brazil and magnified x 400(University of Copenhagen).

If growth conditions continue to be propitious (excess nutrients and favourable weather) *Lyngbya majuscula* can rapidly grow at an exponential rate to what is often referred to as an "algal bloom" (Arquitt and Johnstone 2004; Albert *et al.* 2005; Watkinson *et al.* 2005; Ahern *et al.* 2007; Estrella 2013). To achieve this, *Lyngbya majuscula* goes through four distinct growth phases. These are 1) incipience; 2) rapid expansion; 3) plateau or peak and 4) decline phases (Ahern *et al.* 2007; Estrella 2013). Through these four phases the effects of *L. majuscula* have the potential to negatively affect an ecosystem and the organisms that come into contact with it through ecological pressures (Albert *et al.* 2005; Watkinson *et al.* 2005; Garcia and Johnstone 2006; Osborne *et al.* 2007; Estrella 2013).

1. *Lyngbya majuscula* bloom formation

When conditions are right for the *L. majuscula* to grow it will attach to a substrate or sedentary organism this is the incipient or 'colonisation' growth phase (Albert *et al.* 2005; Paul *et al.* 2005; Pittman and Pittman 2005; Arthur *et al.* 2006; Garcia and Johnstone 2006; Johnstone *et al.* 2007). Once established, fragmentation begins and strands of the *L. majuscula* increase in length (Ahern *et al.* 2008). In this rapid expansion growth phase *L. majuscula* has the capacity for exponential growth and aerial expansion and whilst conditions are still favourable will smother any organism that is vying for sunlight (Garcia and Johnstone 2006; Ahern *et al.* 2007; Martın-Garcıa *et al.* 2014). Mats are formed when the

strands entangle on each other and link up during the plateau or peak phase. These mats can often cover up to 100% of the benthos and be up to 15 cm high (Ahern *et al.* 2007; Estrella 2013; Martın-Garcıa *et al.* 2014).When nutrients are depleted and conditions become unfavourable, oxygen bubbles formed in the *Lyngbya majuscula* mats due to decomposition and breakdown of the cells, cause them to float to the surface. These *Lyngbya majuscula* mats end up onshore or float/migrate to other areas (Ahern *et al.* 2007). The final growth phase is the decline, a massive reduction in biomass and darkness in pigmentation and decomposition occurs that can often lead to anoxic conditions in the water column and costly clean up (Figure 2)(Ahern *et al.* 2007).

2. *Lyngbya* and nutrients

The main contributing factors to the presence of *Lyngbya majuscula* in an ecosystem are nutrients, in particular phosphorus and bioavailable iron and good weather (hot with clear skies) (Hamilton *et al.* 2009; Johnson *et al.* 2010; Agrawal 2012). This gives the initial start that *L. majuscula* needs in order to have permanent seasonal blooms and stay in an ecosystem.

In general cyanobacterial growth, the same as any other photosynthetic organism, is limited by the availability of fertilisers such as nitrogen and phosphorous (Postgate 1987; Ahern *et al.* 2008). Nitrogen is often the limiting nutrient for the potential of a bloom to occur (Postgate 1987; Ahern *et al.* 2008). *L. majuscula* has a distinct advantage in this regard over many other organisms in the same area due to its nitrogen fixing properties and not relying on bioavailable nitrogen in the water column to grow (Paerl *et al.* 1987; Ahern 2004; Ahern *et al.* 2008). With the increase of nutrients from anthropogenic sources the occurrence of this toxic cyanobacterium will likely increase in tropical and sub-tropical waters (Ahern *et al.* 2008). Studies were also carried out in Sentosa Cove, Singapore where *L. majuscula*'s ability to use nutrients in resuspended sediment were tested; biomass was found to increase with higher amounts of nutrients suspended in the water column and it grew faster than competing organisms due to its rapid growth phase (Ng *et al.* 2012).

In Roebuck Bay recent studies have linked lower concentrations of ammonium and phosphorus in areas with maximum *L. majuscula* biomass (Estrella 2013). This negative correlation has been explained as *L. majuscula* initially starting on sediments rich in ammonium and phosphorus and then depleting these nutrients from the sediment through

high growth (Estrella 2013). These findings again associate *L. majuscula* with enriched nutrient sediments. In Roebuck Bay several studies have found nutrient levels above ANZECC/ARMCANZ (2000) water quality guidelines (Vogwill 2003; Estrella 2013). A recent study has pointed out the potential risk of eutrophication, which is known as a driving force for algal blooms (Figure 3)(Estrella 2013). Therefore the main recommendation proposed has been to reduce the input of nutrients (most notably phosphorus) into Roebuck Bay (Estrella 2013).

3. *Lyngbya* and temperature/solar radiation

Temperature has been found to have a major impact on the probability of a *L. majuscula* bloom occurring. A major indicator of the likelihood of a bloom was found to be when the mean monthly air temperature is around 26°C, which is only about 1 °C less than water temperature in Deception Bay, Queensland, Australia (Hamilton *et al.* 2009). In Roebuck Bay warm temperatures that are overall higher than in Deception Bay in December have been identified as one of the triggers of *L. majuscula* blooms (Estrella 2013).

In regard to solar radiation, light wavelength and intensity, *L. majuscula* possesses a complementary chromatic adaptation (CCA) which allows it to alter pigment levels so as to optimize the capacity for photosynthesis and nitrogen fixation (Jones *et al.* 2009). It is capable of aerobic nitrogen fixation during the day and is limited by low light environments (Jones 2007). *L. majuscula* also has an effective adaptation mechanism to withstand ultraviolet radiation, causing an increase in thickness of the mucilaginous layer allowing it to absorb the radiation and survive for prolonged periods of time under harsh light conditions (Mandal *et al.* 2011; Pessoa 2012). Therefore *Lyngbya majuscula* is an opportunistic cyanobacterium which can adapt to changing light and radiation to efficiently fix nitrogen and grow under harsh conditions (Hamilton *et al.* 2007; Jones 2007; Pessoa 2012). Although high temperatures have been found as a potential trigger of *Lyngbya* blooms (Johnson *et al.* 2010; Paerl and Paul 2012).It was also recorded at the Peel-Yalgorup Ramsar site in the south-west of Western Australia where the temperatures are in general lower (Hale and Butcher 2007).

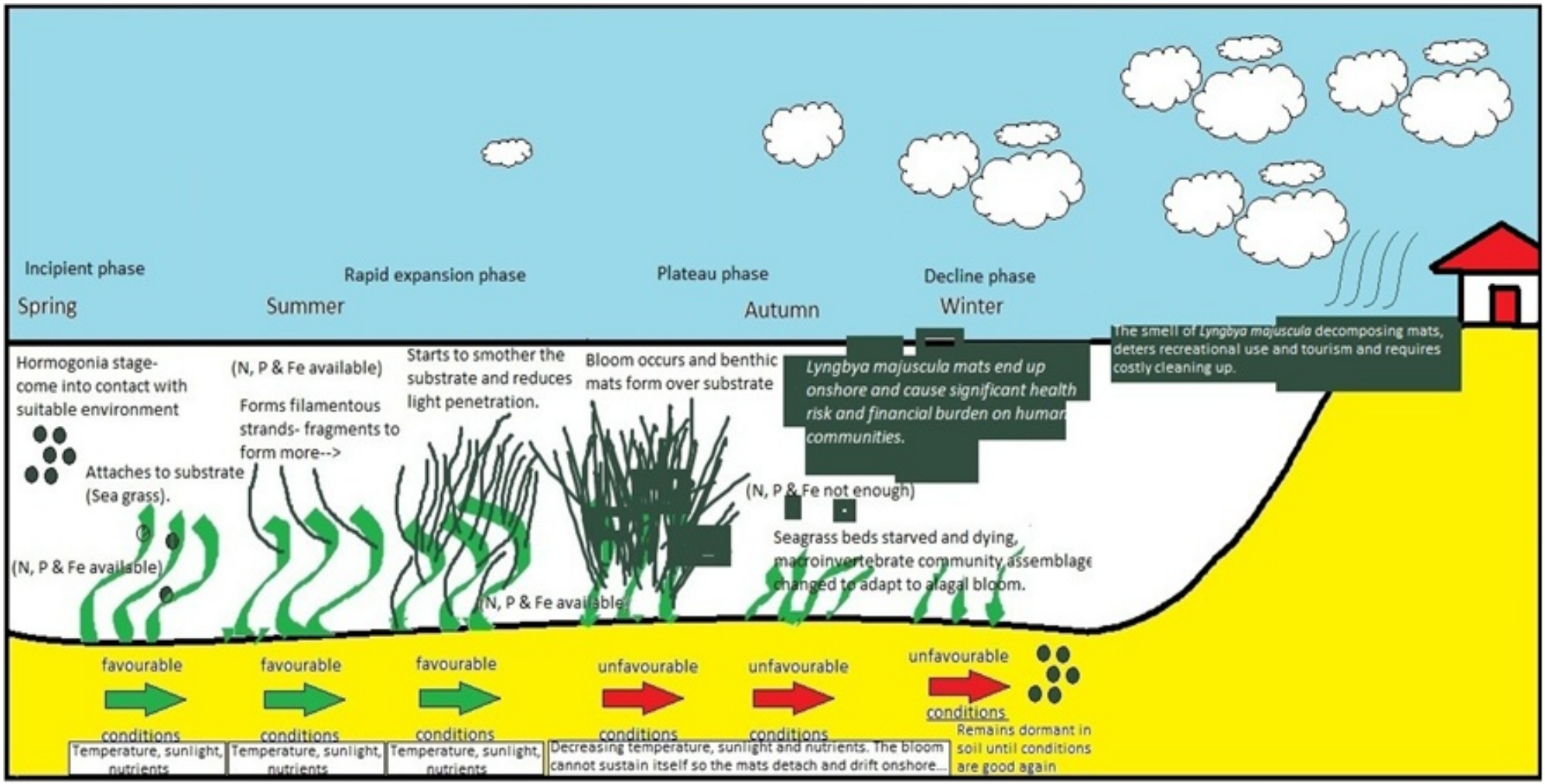

Figure 2. *Lyngbya majuscula* bloom life cycle reproduced from Ahern et al. 2008 and Estrella 2013.

4. *Lyngbya majuscula* bloom effects

Lyngbya blooms can lead to major ecosystem changes and have been known to **a**) affect oxygen levels in the water column (Ahern *et al.* 2007; Martinetto *et al.* 2010; Agrawal 2012; O' Neil *et al.* 2012), **b**) inhibit seagrass growth due to reduction in light and smothering (Figure 3)(Stielow and Ballantine 2003; Kuffner and Paul 2004; Garcia and Johnstone 2006), **c**) reduce macroinvertebrate diversity and density (Estrella *et al.* 2011) due to oxygen depletion, toxic compounds and reducing suitable habitats (Capper *et al.* 2005; Ferris and Bongers 2006; Ahern *et al.* 2007; Paul *et al.* 2007; Geange and Stier 2010; Estrella *et al.* 2011; Estrella 2013; Taylor *et al.* 2014), **d**) affect marine vertebrate food pathways (such as sea turtles and dugongs) caused by accidental feeding on the toxic cyanobacteria (Arthur *et al.* 2006; Arthur *et al.* 2006; Arthur *et al.* 2008; Baumberger 2008; Ismael 2012), and **e**) have side effects on shorebirds and human health (Grauer and Arnold 1961; Codd *et al.* 1999; Watkinson *et al.* 2005; Ahern *et al.* 2008; Osborne *et al.* 2008; Estrella 2013).

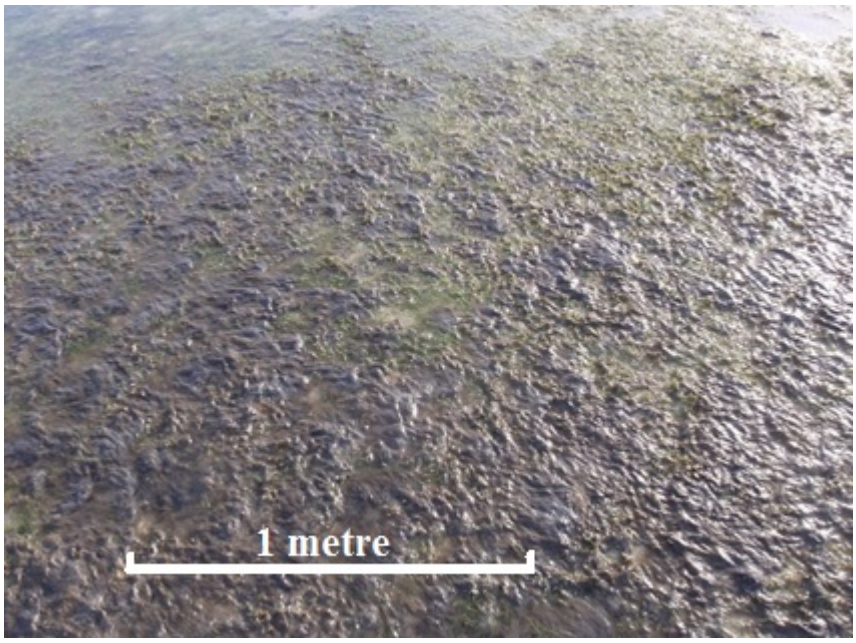

Figure 3. *Lyngbya majuscula* covering a seagrass meadow in Roebuck Bay, Broome on February 25, 2012. (Photos T. A. de Silva).

Furthermore these blooms can also pose a financial burden on the human communities that are affected by them (Watkinson *et al.* 2005; Ahern *et al.* 2008). *L. majuscula* blooms have had significant impacts on commercial fish catches due to decreased feeding and nursery habitats (seagrass and corals) and the costs of cleaning up fouled trawl nets and buoys (Dennison and Abal 1999; Dennison *et al.* 1999; Hamilton *et al.* 2009). Local communities

have also been affected through lost tourism and the need to clean-up mats washed up on the beach (Albert *et al.* 2005; Ahern *et al.* 2007). According to Moreton Bay Regional Council's *Lyngbya* Management Strategy (2011); Caboolture Shire Council's clean-up to remove *Lyngbya majuscula* washed onto local beaches cost more than $725,000 over four summers.

L. majuscula can become a persistent problem for ecosystems and communities partly due to its life history. The formation of floating benthic mats due to gas bubble build up during the decomposition stage allows this cyanobacteria to travel to new areas through current movement (Pittman and Pittman 2005; Johnstone *et al.* 2007). This combined with the ability to stay in a dormant cyst stage for a prolonged period of time within the sediment makes it very difficult to eliminate once it has been introduced (Ahern *et al.* 2008; Paerl and Paul 2012). The prevention of potential *L. majuscula* establishment would be the best option but it is extremely difficult to regulate all the available pathways *Lyngbya majuscula* has into an environment(Albert *et al.* 2005; Hamilton *et al.* 2009; M.B.R.C 2011; Estrella 2013).

5. *Lyngbya* defence and survival mechanisms

In southeast Florida, USA, *Lyngbya* species were found to deter generalist herbivores (Capper and Paul 2008); however, some species can tolerate and will selectively eat *Lyngbya.* Some of these species are *Stylocheilus striatus* (sea hare), *Haminoea antillarum* (gastropod) and *Bulla striata* (gastropod) (Capper and Paul 2008). The interactions of these grazing species with *L. majuscula* and two other species of *Lyngbya*, *L. polychroa* and *L. confervoides* was tested to find out feeding preference (Capper and Paul 2008). Grazing is an important ecological population control mechanism (Capper and Paul 2008).Within the three tested invertebrate species, *L. majuscula* was found to be consumed in the smallest amount. This would suggest *L. majuscula* is the least appealing of the three and would deter grazers more than the other species of *Lyngbya*.

There have been studies related to *Lyngbya majuscula* growing on guano from seabirds on a helicopter pad that had caused localised blooms in the Hardy reef area (Great Barrier Reef), showing the potential for *Lyngbya majuscula* to utilise nutrients when given an opportunity and evidence the cyanobacteria can migrate to other areas (Ahern *et al.* 2008).

6. Negative effect of *Lyngbya* on other organisms

6a. *Lyngbya* effects on oxygen levels

During a bloom, *L. majuscula* has a negative effect on meiofauna induced by oxygen depletion within the sediment (Garcia and Johnstone 2006). Consequently, due to the role meiofauna plays in sediment remineralisation, *Lyngbya majuscula* is also limiting available inorganic substances to other photosynthetic organisms such as seagrass (Garcia and Johnstone 2006). This in turn changes the natural pathways and ratios of inorganic compounds in the ecosystem and can potentially lead to a permanent loss of several native species susceptible to these effects, such as, polychaetes and nematodes (Garcia and Johnstone 2006). This can significantly affect the biodiversity of a localised area.

Anoxic/hypoxic events occur during the night when cyanobacterial respiration consumes oxygen from the water (Garcia and Johnstone 2006; Ahern *et al.* 2007; Albertin 2009; Martinetto *et al.* 2010; Paerl and Paul 2012). This can be reversed by production of oxygen by photosynthesis during the day (Ahern 2004; Martinetto *et al.* 2010; Paerl and Paul 2012). If there are consecutive cloudy days then the uptake of oxygen can be more than production and this can turn the system anoxic (Garcia and Johnstone 2006; Johnstone *et al.* 2007; Martinetto *et al.* 2010; King 2012).

Hypoxic and anoxic events affect the survival of any organisms (i.e. fish and shellfishes)(Pittman and Pittman 2005; Paerl and Paul 2012). A *Lyngbya* bloom might affect invertebrates living in the sediment by changing the chemical environment that exists within the sediments (Garcia and Johnstone 2006). This affects different invertebrates in different ways; copepods have a very low tolerance to anaerobic conditions and can only be found in the oxygenated layers of sediment (Garcia and Johnstone 2006). Meiobenthos burrowing is known to enhance the distribution and level of oxygenation throughout different sediment depths but if the meiobenthos is being detrimentally affected by *L. majuscula* and the sediments become less oxidised (Garcia and Johnstone 2006).

6b. Seagrass and coral

Impacts of *Lyngbya majuscula* blooms include smothering and overgrowth of intertidal benthic communities (Stielow and Ballantine 2003; Watkinson *et al.* 2005). In Guam, the effects of *L. majuscula* on larval survival of the corals; *Acropora surculosa* and *Pocillopora damicornis* were tested (Kuffner and Paul 2004). Larvae in both species were found to avoid *L. majuscula*. The coral larvae also opted for areas with the lower *L. majuscula* densities when avoiding was not possible (Kuffner and Paul 2004). This overall significantly lowers recruitment success of these corals (Kuffner and Paul 2004).

L. majuscula can adversely affect seagrass meadows via smothering and changing nutrient pathways (Paul *et al.* 2005; Ahern *et al.* 2007; Johnson *et al.* 2010). Its thick growth form can smother and block out light when it attaches to seagrass which can slow or stop photosynthetic processes (Pittman and Pittman 2005; Ahern *et al.* 2007). Lyngbya can also use up and limit nutrients found in the water column, such as nitrates, nitrites and phosphates, that seagrass would usually uptake (Garcia and Johnstone 2006). This effectively starves the seagrass of food and causes a decline in the health and abundance of these meadows (Dennison *et al.* 1999; Albert *et al.* 2005; O' Neil *et al.* 2012). The effects on corals and seagrass then carry onto the macroinvertebrates and vertebrates that inhabit those communities (Dennison *et al.* 1999; Garcia and Johnstone 2006; Ahern *et al.* 2007; Estrella 2013).

6c. Macroinvertebrates

Macroinvertebrates have been shown to be adversely affected by *L. majuscula* in varying degrees, from species to a community level, with different species being more affected than others (Estrella 2013). *Lyngbya* can affect dissolved oxygen, sediment grain size, remineralisation and competition and food availability in a complex benthic habitat (Giere 1993; Steyeart *et al.* 2003). A marked amount of sediment remineralisation and support for higher trophic pathways is due to the meiofaunal community (nematodes, copepods and polychaetes), and because of their short life cycles/quick generation times these organisms can give a good indication of environmental disturbances (Garcia and Johnstone 2006). Garcia and Johnstone (2006) found a 74% decrease in the amount of invertebrates during a bloom. Species that are more affected in the presence of a bloom are nematodes, copepods

and isopods (Garcia and Johnstone 2006; Estrella 2013). The toxins from *Lyngbya majuscula* would not likely be transported as deep as 20 cm in the sediment during the bloom so meiofauna that can dig deeper than this (such as polychaetes) may be less affected (Garcia and Johnstone 2006).

During a *Lyngbya* bloom, large amounts of material on benthic habitats can create a sub-optimal environment which reduces the abundance of prey and physically interfere with feeding behaviour (Raffaelli *et al.* 1998). Bivalves decrease in the presence of blooms and their filtering capacity and feeding decline as a large filamentous mass stops zooplankton from feeding on phytoplankton (Raffaelli *et al.* 1998; Paerl and Otten 2013). The sea hare *Stylocheilus striatus*, will selectively feed on *Lyngbya majuscula* and the predatory nudibranch, *Gymnodoris ceylonica* will predate on the sea hare, forming a linear food chain (Geange and Stier 2010). This means that the presence of *Lyngbya* can change the natural food web of an ecosystem with the combination of lowering certain taxa and promoting others (Geange and Stier 2010; Estrella *et al.* 2011; Estrella 2013).

In Roebuck Bay the presence of high densities of *Lyngbya majuscula* (mean biomass >300 g DM/m²) had shown significant effects on the composition, abundance and diversity of benthic macroinvertebrates (Estrella 2013). Brittle stars (ophiurids) were found to decrease along with other macroinvertebrates (Estrella *et al.* 2011). Polychaetes in this area were found to increase during a bloom but this could also be seasonal variation; where higher densities occur in the wet season in a tropical tidal flat (Metcalfe and Glasby 2008). Some groups of gastropods such as Hamioeidae and Bullidae families (Estrella, S.M. personal communication, May 7, 2014) also increased mainly due to feeding on *L. majuscula* and being resilient to its anoxic/hypoxic effects during low tide (Estrella 2013).
The taxa Sipunculidae (Peanut worm) only appeared during blooms and was found in the thousands per square metre in the presence of in high densities of *L. majuscula* (>300 DM/m²), this in turn changed the food web and feeding habits of shorebirds in Roebuck Bay (Estrella *et al.* 2011). This is most probably due to the sipunculids resistance to the *Lyngbya majuscula* toxins, asexual reproduction via transverse fission, a tolerance of hypoxic conditions and very short trochophore larval stage (1-30 days) which could possibly be spent among sea grass or trapped within the *Lyngbya majuscula* strands so when it reaches adult form colonies appear in high numbers (Barnes 1982; Langenbuch and PÖrtner 2004; Vaquer-Sunyer and Duarte 2008).

6d. Vertebrates

In the King's Bay, Florida ecosystem Debrimoaplysiatoxin (a toxin produced by *L. majuscula*) was found in manatee (*Trichechus manatus* ssp. *latirostris)* dorsa, as well as, ulcerative dermatitis on manatees known to feed on the surrounding *Lyngbya majuscula* (Harr *et al.* 2008). *Lyngbya majuscula* has also been linked to tumours in marine turtles (Arthur *et al.* 2006; Arthur *et al.* 2008; Capper *et al.* 2013). As mentioned earlier, vertebrates can be indirectly impacted by *L. majuscula* such as modifications on shorebirds foraging behaviour through the induced changes on macroinvertebrate communities (Estrella *et al.* 2011; Estrella 2013).

7. *Lyngbya* toxins and human effects

Lyngbya majuscula has been the focus of many studies and has been found to be a particularly resilient and adaptable cyanobacterium. Studies in Hawaii, Okinawa and Florida showed that "swimmer's itch" (caused by aplysiatoxins and Lyngbyatoxin-a) had affected people swimming in coastal areas that had *L. majuscula* present (Grauer and Arnold 1961; Hashimoto *et al.* 1976; Codd *et al.* 1999).

The pantropical genus *Lyngbya* is an extremely rich source of bioactive secondary metabolites and consists of 35% of all reported cyanobacterial natural products (over 240 compounds) (Paul *et al.* 2007; Jones *et al.* 2011). Of these, over 76% are attributed to the one species *L. majuscula*, making it the most prolific secondary metabolite producer of all the species within the genus (Jones *et al.* 2011). *L. majuscula* contains toxins such as dermatoxic aplysiatoxins and Lyngbyatoxin-a that can cause asthma, allergies, dermatitis (or "Swimmer's itch") and eye irritation in humans (Grauer and Arnold 1961; Hashimoto *et al.* 1976; Codd *et al.* 1999; Osborne *et al.* 2001; Osborne *et al.* 2007; Osborne *et al.* 2008; Taylor *et al.* 2014). *Lyngbya majuscula* studies usually focus on its effects on human health or on the health of an ecosystem with focus on a key vertebrate group such as a turtles, manatees/dugongs or shorebirds highlighting how significant this marine species is in affecting any organism it comes in to contact with (Arthur *et al.* 2006; Arthur *et al.* 2008; Harr *et al.* 2008; Estrella *et al.* 2011).

8. *Lyngbya* within Australia

The first identified bloom of *L. majuscula* within Australia was in Deception Bay (Moreton Bay), Queensland in 1996 (Dennison *et al.* 1999). Since then many more records have been made more widely within Australia and Queensland (Table 1). Nuisance blooms tend to occur seasonally in Australian waters showing the opportunistic nature of *Lyngbya* (Dennison *et al.* 1999; Albert *et al.* 2005; Estrella 2013). This indicates that *L. majuscula* is an opportunistic organism which will adapt to and remain in a system through a dormant cyst stage (Potts 1994; Nagle *et al.* 1996; Albert *et al.* 2005; Martinetto *et al.* 2010; Jones *et al.* 2011). *L. majuscula* blooms have increased in both frequency and severity in Moreton Bay, Queensland, and other parts of Australia in conjunction with an increase in nutrients across coastal and estuarine communities (Albert *et al.* 2005; Hale and Butcher 2007; Ahern *et al.* 2008; Estrella 2013).

Table 1. Australian sites where *Lyngbya majuscula* has been recorded and the substrates it was found attached to.

State	Area	Site	Year	Substrate or photosynthetic organism	Reference
New South Wales	Queensland	Moreton Bay	1996	seagrass	(Dennison *et al.* 1999)
		Eastern Banks	2000	seagrass	(Albert *et al.* 2005)
		Adams Beach	2001	seagrass	(Albert *et al.* 2005)
		Horseshoe Bay	2001	seagrass	(Albert *et al.* 2005)
		Wellington Point	2002	seagrass	(Albert *et al.* 2005)
	Southern Queensland	Hervey Bay	1999	coffee rock/seagrass	(Dennison *et al.* 1999)
		Fraser Island	1999	coffee rock/seagrass	(Dennison *et al.* 1999)
	Central Queensland	Shoalwater Bay	2002	seagrass	(Albert *et al.* 2005)
		Whitsunday Islands	2001	seagrass	(Albert *et al.* 2005)
		Hardy Reef	2001	coral	(Ahern *et al.* 2007)
		Scawfell Isand	2004	coral	(Albert *et al.* 2005)
		Keppel Islands	2002	coral/ seagrass	(Albert *et al.* 2005)
	Northern Queensland	Hinchinbrook Island	1999	seagrass	(Dennison *et al.* 1999)
		Cape Kimberley	1999	coral	(Dennison *et al.* 1999)
Western Australia	North-Western Australia	Rowley Shoals	2009	coral	(Huisman *et al.* 2009)
		Scott Reef	2009	coral	(Huisman *et al.* 2009)
		Seringapatam Reef	2009	coral	(Huisman *et al.* 2009)
		Roebuck Bay, Broome	2005	seagrass	(Deeley 2009)
	South-Western Australia	Peel Inlet- Lake Goegrup	1999	Seagrass	(Dennison *et al.* 1999)
		Rottnest Island	2008	Seagrass	(Cambridge 2008)

9. Roebuck Bay, Broome, Western Australia.

Broome, northern-Western Australia has a seasonal, tropical climate and consists of a warm-dry season (May-November) and a hot-wet season (December-April) (Estrella *et al.* 2011). The study site is located in Roebuck Bay, Broome and has been a Ramsar wetland since 1990 and hosts one of the richest macroinvertebrate mudflats in the world (Piersma *et al.* 1998; Rogers *et al.* 2003). This is also one of the most significant and renowned shorebirds habitats in Australia(Piersma *et al.* 1998; Rogers *et al.* 2003). However blooms of *L. majuscula* have been observed since 2005 during the hot-wet season and a recent study has found that these blooms can have a significant effect on the macrobenthos community assemblage and shorebirds foraging behaviour (Estrella *et al.* 2011; Estrella 2013). Roebuck Bay has potential nutrient input from the wastewater treatment plant and urban sprawl; such as the golf course and Dampier Creek (Estrella 2013). The continued occurrence of these blooms in Roebuck Bay during the wet season suggests that the combination of nutrient inputs, rainfall run-off (which increases bioavailable iron) and high water temperatures are beneficial for the growth of *Lyngbya majuscula* (Estrella *et al.* 2011).

Although, there have been many studies done on *L. majuscula;* the studies are usually centred on the larger tasks such as management strategies or the effects on larger vertebrates (turtles, shorebirds, dugongs, etc.); the direct influence it has on macroinvertebrates has not been fully explored. Roebuck Bay in Broome, Western Australia was chosen for previous studies and re-occurrence of *L. majuscula*.

Project rationale

Previous studies of Roebuck Bay before (November), during (February) and after (May) a *Lyngbya majuscula* bloom showed macroinvertebrate assemblages can be negatively affected during a bloom (Estrella *et al.* 2011; Estrella 2013; Estrella 2013). Yet a gap in knowledge regarding the direct effects on macroinvertebrates remains.

This project aims to study the effect of an artificial bloom of *L. majuscula* on macroinvertebrate diversity and abundance, replicating the natural growth phase of a bloom. This was carried out by sampling macroinverterbates every six days over the period of time leading up to a bloom and the decomposition of it (February-April). Fixed quadrats were used to keep the *Lyngbya majuscula* biomass in the impact quadrats while keeping the control

quadrats completely free of *Lyngbya majuscula*. Water quality measurements (nutrients, dissolved oxygen, pH and temperature) for any abiotic changes in the water column and mud cores for changes in the sediments were also taken.

This allowed for a closer and more detailed study of macroinvertebrate abundance and species richness in the presence of *L. majuscula* compared to assemblages in a control treatment (without any *Lyngbya majuscula).*

Hypothesis

Macroinvertebrate abundance and diversity, sediment and water assessments were used to test the following hypothesis: *L. majuscula* has a negative impact on both abundance of the macroinvertebrates and the species richness during a simulated bloom event.

Materials & Methods

1. Study Site

The study site, which is approximately 400 metres offshore from Simpson's Beach (17°59'00"S 122°12'59"E) near the Port of Broome in Roebuck Bay (Figure 4) ,was chosen due to previous studies (involving *L. majuscula* mapping and biomass studies) in the bay (Estrella *et al.* 2011; Estrella 2013; Estrella 2013). Simpson's Beach is characterised by sandy sediments, high sand dunes and the existence of seagrass meadows (Estrella 2013). The initial stages of a *L. majuscula* bloom were reoccuring regurlarly at the study site in December each year. With favourable conditions the extent of *Lyngbya* would expand northward towards Dampier Creek during the wet season. If conditions continue a full bloom around February/March could extend to Crab Creek (Estrella *et al.* 2011; Estrella 2013).

The *Lyngbya* bloom could be due to the number of nutrient input sources (Figure 5) surrounding the area including the golf course and storm water run-off (Estrella 2013), activities from the port, waste water treatment plant (that dischargees waste water effluent directly into the bay) (Gunaratne 2014).In correspondence with these observations, a sampling program was carried out during the lead up to an algal bloom and gradual decline over a period of 2 months (February-April).

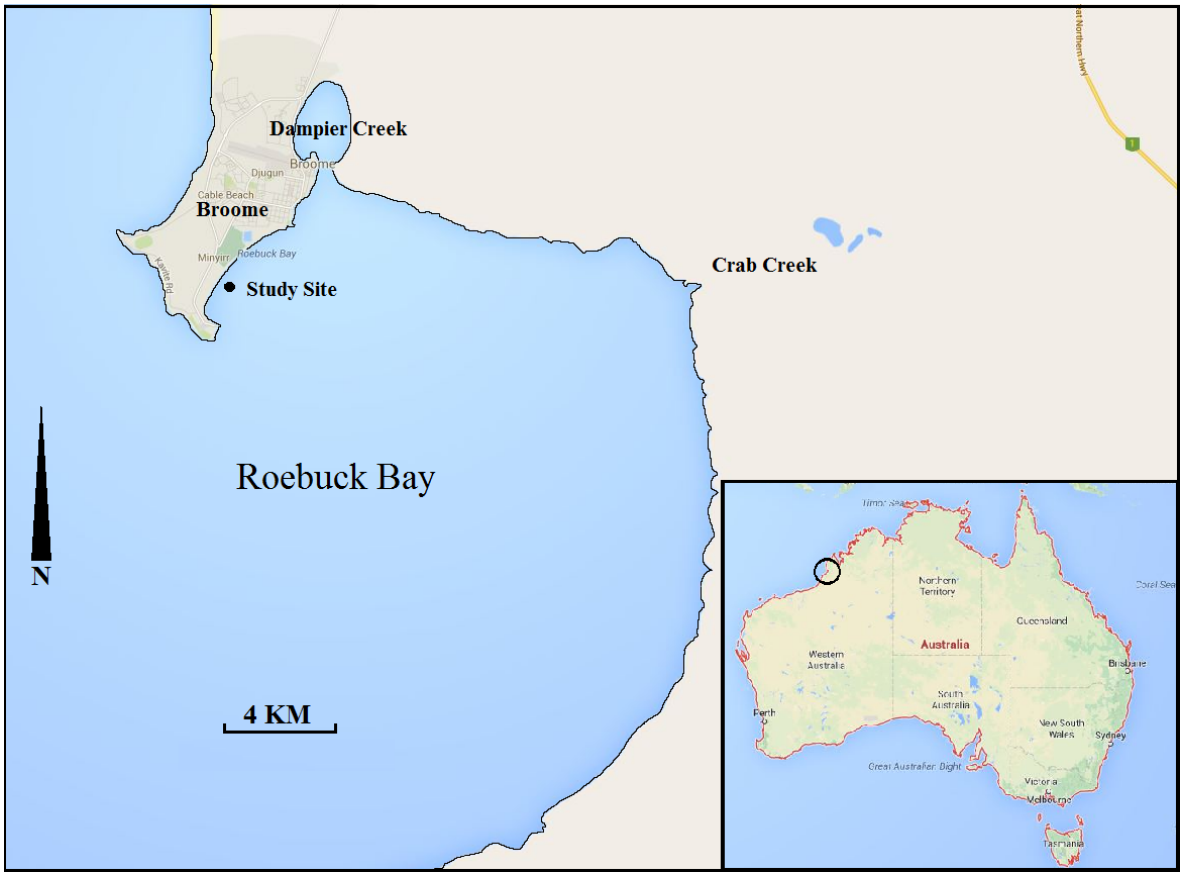

Figure 4. Map of Roebuck Bay, in North Western Australia, with the study site indicated (17°59'23.20"S, 122°13'0.00"E) (Google Maps, Microsoft Paint).

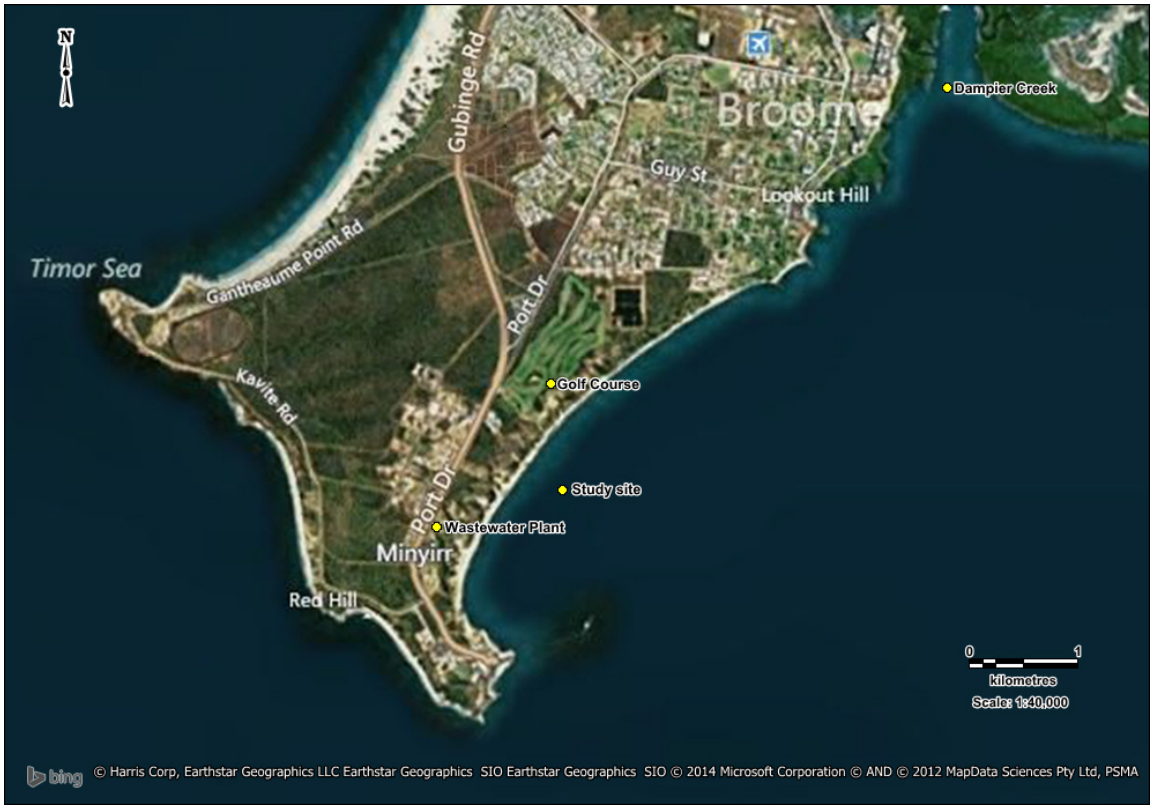

Figure 5. Study site and potential nutrient sources highlighted, Roebuck Bay, Western Australia (Bing Maps).

2. Study design

The study was designed to test the direct effects of *Lyngbya majuscula* on macroinvertebrates and whether any trends could be seen in the lead up to the artificial bloom in abiotic variables, such as dissolved oxygen, pH, temperature and nutrients. To test this, macroinvertebrate assemblages were kept within an enclosed area to mitigate previously recorded macroinvertebrate movement away from *L. majuscula* (Baumberger 2008; Geange and Stier 2010; Gilby *et al.* 2011). Fixed quadrats were constructed to allow natural water flow and processes to occur while containing any macroinvertebrate assemblages (see page 9 for more information on Hypothesis). Abundance and species richness were the variables used to test the hypothesis of *L. majuscula* negatively affecting the macroinvertebrate assemblages. Three replicates (quadrats) were set up for keeping in *L. majuscula* (impact) and three replicates (quadrats) were set up for excluding *L. majuscula* (control). To test if there were any effects of the fixed quadrats on the macroinvertebrate abundance and species richness, a third type of quadrat was constructed (random controls). These were not fixed in place like the control and impact quadrats. These random controls were set up for comparison studies with three replicates (quadrats). Samples were taken every six days at low tide to avoid the effects of tide on macroinvertebrate distribution (Escapa *et al.* 2004; McLachlan

and Brown 2006)(Table 2). Not all parameters were sampled for the entire duration of the study with water quality (D.O., pH, Temperature and nutrients) being recorded for the first five samples (Day 0-24).

Table 2. Sampling program for *Lyngbya majuscula* macroinvertebrate, sediment, water nutrients and water quality sampling.

Sampling Period	Day	No. sites	No. samples	Time of day sampled	Samples collected	Tidal data (Taken from BOM)
28 Feb 2013	0	9	27	0600- Including set up of quadrats.	macroinvertebrate, sediments, water quality.	L: T- 0624, 0.97 m H: T-1119, 9.18 m
6 March 2013	6	9	27	0800	macroinvertebrate, sediments, water quality.	L: T- 0919, 3.94 m H: T- 1543, 7.28 m
12 March 2013	12	9	27	0600	macroinvertebrate, sediments, water quality.	L: T- 0513, 1.25 m H: T- 1106, 9.60 m
18 March 2013	18	9	27	0700	macroinvertebrate, sediments, water quality.	L: T- 0754, 2.48 m H: T- 1351, 8.61 m
24 March 2013	24	9	27	1500	macroinvertebrate, sediments, water quality.	L: T- 1531, 3.83 m H: T- 2133, 7.56 m
1 April 2013	32	9	27	0700	macroinvertebrate, sediments	L: T- 0758, 2.35 m H:T- 1356, 8.91 m
8 April 2013	39	9	27	1500	macroinvertebrate, sediments	L: T- 1555, 2.57 m H: T- 2151, 8.53 m
14 April 2013	45	9	27	0600	macroinvertebrate, sediments	L: T- 0644, 1.71 m H: T- 1232, 9.47 m
22 April 2013	53	9	27	1400	macroinvertebrate, sediments	L: T- 1442, 3.99 m H: T- 2044, 7.20 m

This was implemented by choosing an intertidal area that would be exposed during low tide even during the smallest of neap tides to allow consistent sampling. The fixed quadrats were placed in a randomised block design within 50 metres of the minimum low tide line and dug into the sediment to a depth of 8 to 15 cm, taking care to minimise disturbance to the sediment (de Goeij *et al.* 2003; Ahern *et al.* 2008; de Goeij *et al.* 2008). These quadrats remained in the same place for the duration of the study. The random control quadrats were placed haphazardly each sampling period to give a representation of natural abundance and richness with no containment (Figure 6).

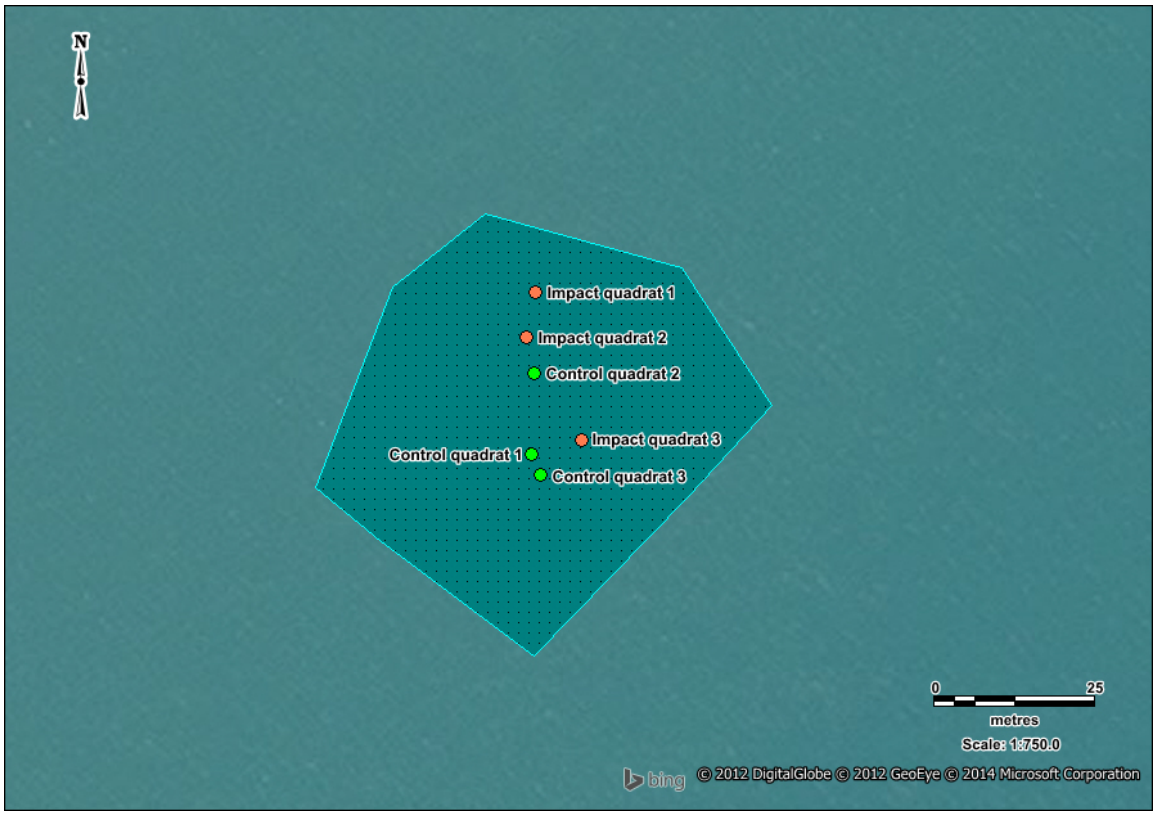

Figure 6. Impact and control fixed quadrat and all random control unfixed quadrats (81 over the course of the study) forming the shaded area (17°59'23.20"S, 122°13'0.00"E), Roebuck Bay, Western Australia (Bing Maps).

3. Quadrat construction

In situ quadrats were installed from the 28th of February til the 22nd of April 2013 at the study site (Figure 5) to regulate the amount of *Lyngbya majuscula* within each replicate during the period of this study. Three fixed impacts n=3 (or quadrats with *Lyngbya* placed within them) and three fixed controls n=3 (or quadrats with *Lyngbya* kept out of them) were installed using simple random design. Quadrats were (62 cm x 42 cm x 31 cm. L x W x H) in volume (Figure 7). Three unfixed random control quadrats, that changed position each sampling and were chosen using simple random sampling (Yates *et al.* 2008). A quadrat outline (42 cm x 62 cm) was used for this unfixed random control quadrats (Figure 10).

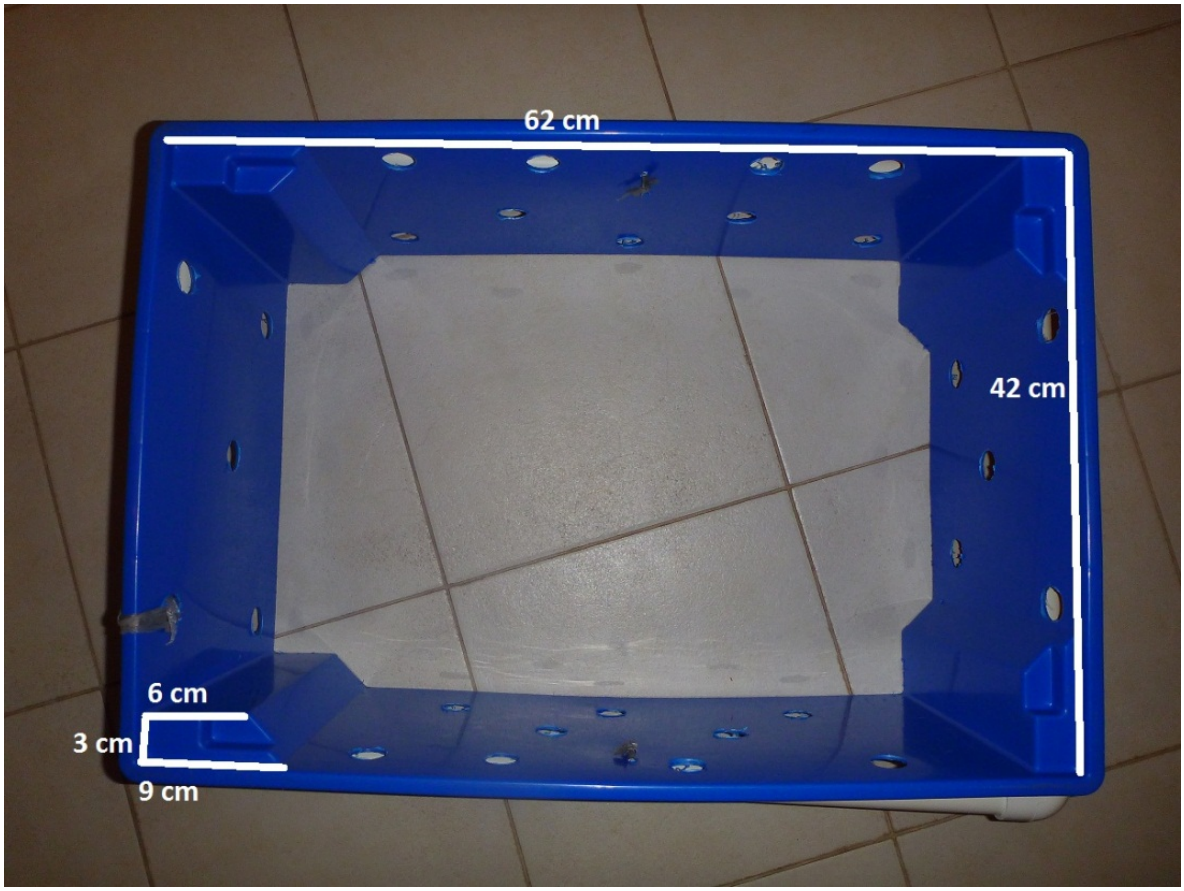

Figure 7. Quadrat view from above with dimensions. (Photos T. A. de Silva).

The fixed quadrats had nine 2.5 cm diameter holes drilled along the length and five 2.5 cm diameter holes drilled along the width to allow for water movement (Ahern *et al.* 2008). 1 mm diameter fly wire wrapped around the circumference and over the top of the quadrat and was kept in place via cable ties (Figure 8). The design allowed for water movement while keeping in macroinvertebrates and *Lyngbya* for the impacts, it also allowed to keep out any

Lyngbya in the control quadrats. Each quadrat was labelled as being part of a study by Department of Parks and Wildlife and the Yawaru Rangers.

Figure 8. Quadrat covered with mesh to contain macroinvertebrates and keep in or out *Lyngbya majuscula*. (Photos T. A. de Silva).

4. Lyngbya majuscula biomass and bloom simulation.

A natural bloom did not occur in Roebuck Bay during the period of this study. To find out the effects of *L. majuscula* on macroinvertebrates an artificial bloom was created using data gathered from biomass surveys undertaken at this site from Estrella (2013) from February 2010 until April 2012 as a reference. Photos of quadrats taken from the previous study were used to compare and simulate similar amounts for the different stages and biomass (using grams of dry mass per metre squared, g DM/m^2) of a bloom. These amounts were then placed accordingly (using sterile gloves to avoid contact) within the impact treatment for the purpose of this study (Figure 9)(Estrella 2013). The quadrats were sampled for macroinvertebrates, sediment and water quality every sixth day after the initial sampling. Initially, no *Lyngbya* was present in all the quadrats. Day 6 of the artificial bloom involved including very low

amounts of Lyngbya (<10 g DM/m²) and was implemented after the first sampling. In a period of 24 days and 5 sampling periods the *Lyngbya majuscula* biomass in the impact quadrats increased systematically from 0 g DM/m² to over 300 g DM/m², the mean maximum *Lyngbya* biomass observed previously at Roebuck Bay (Estrella *et al.* 2011; Estrella 2013; Estrella 2013). The *Lyngbya* used in quadrats was collected the previous day. The collected *Lyngbya* was cleaned using sea water to remove any foreign macroinvertebrates.

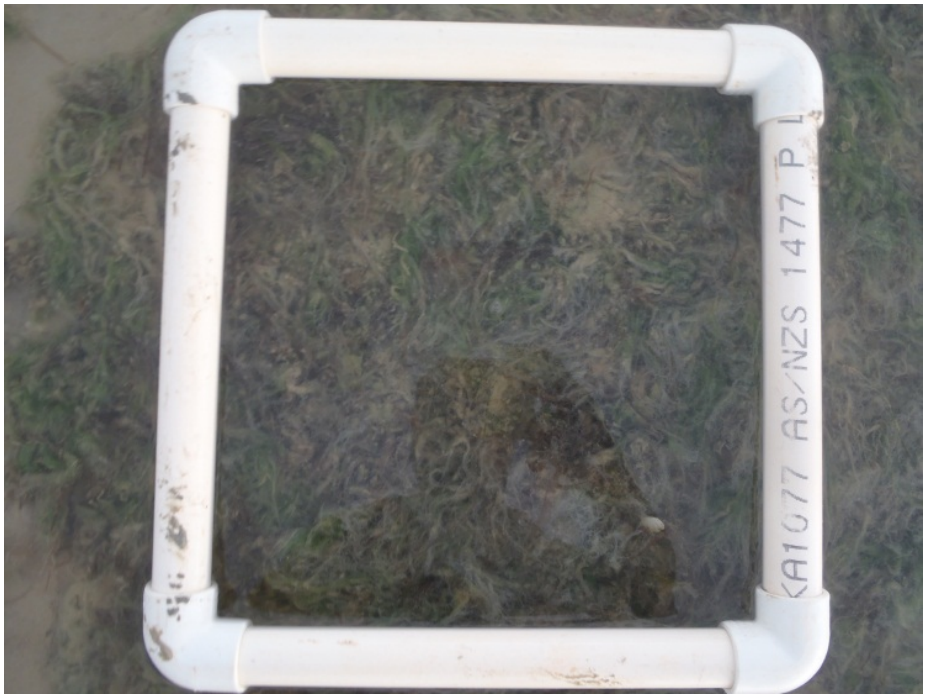

Figure 9. Quadrat from Estrella 2013 study with bloom biomass (> 300g DM / m²) used to imitate artificial bloom for this study. Taken from the same area. (Photos T. A. de Silva).

5. Benthic invertebrate diversity and abundance

To evaluate whether the diversity and abundance of benthic invertebrates varied between the impact and control quadrats samples were taken from all the replicates of each of the treatments every 6 days from the 28th of February, 2013 til the 22nd of April, 2013. Samples were collected by benthic coring using Terumo 60 mL syringe with the adaptor removed; dimensions were 40 mm in diameter and 150 mm deep.

Three mud cores were taken for diversity and abundance and a fourth for sediment grain size analysis per quadrat (Figure 10). Each quadrat was photographed from above to show *Lyngbya* coverage and the addition more *Lyngbya* biomass within the quadrat if needed to simulate a natural bloom biomass (Estrella 2013).

The samples were frozen at – 10 ° C for analysis later in the laboratory. Once thawed each sample was sieved with a 0.5 mm sieve. Each sample was then processed using a stereomicroscope (between 10 and 22 x magnification). All individual macroinvertebrates were removed, identified to family level, and abundance of each family recorded for each quadrat. Family level taxonomy has previously shown enough sensitivity to detect changes in soft bottom assemblages (Bertasi *et al.* 2009). Once each sample had been processed for abundance and richness the samples were kept in 70% ethanol for possible future use and further identification.

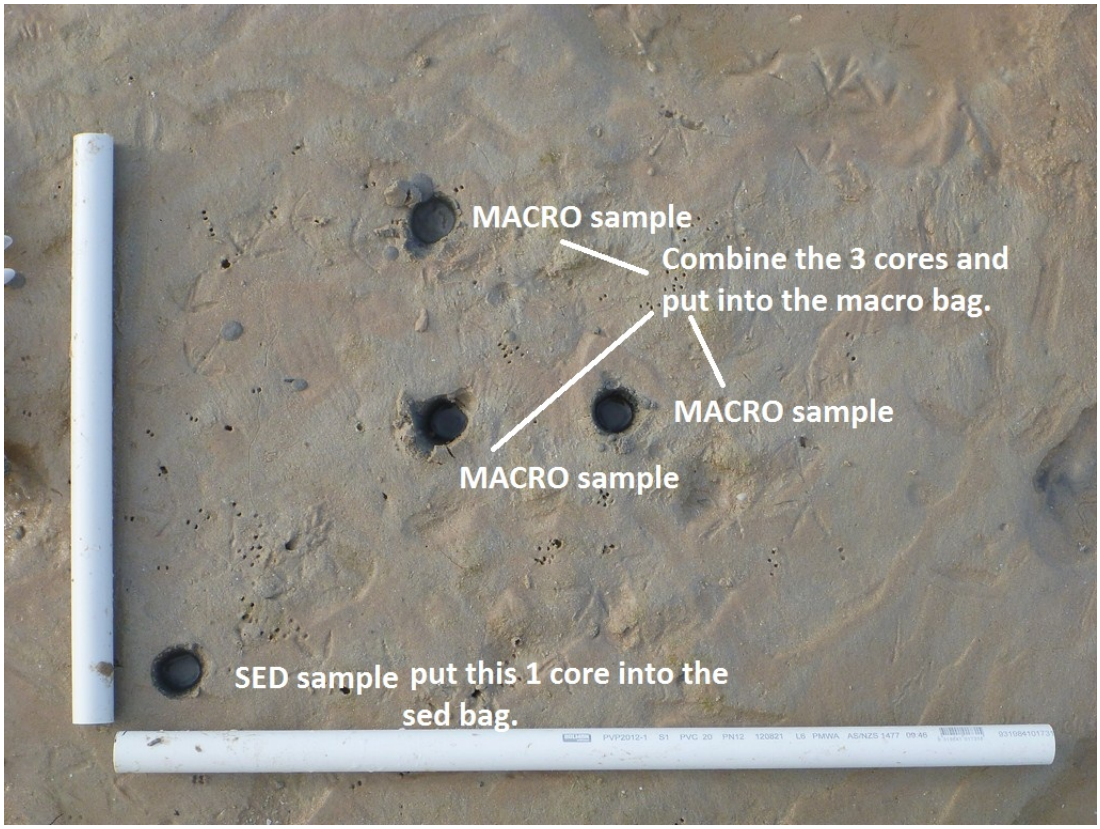

Figure 10. Demonstration of collection of macroinvertebrates and sediment sample, 150 mm deep and 40 mm in diameter sediment cores were taken and formed each of the cavities seen.

6. Sediment grain-size

Samples taken for sediment grain-size analysis were collected using modified methodology from a previous study (Van Keulen and Borowitzka 2003). Using the same modified Terumo 60 mL syringe (length 150 mm, diameter 40 mm) utilised for macroinvertebrate sampling, sediment was collected from the surface to a depth of approximately 150 mm. The sediment sample was released into a plastic bag for storage by pushing the plunger top all the way into the barrel of the syringe. One core was taken for each quadrat for sediment grain-size analysis after the three for the macroinvertebrate sampling (Figure 10). The collected material was dried in the sun over the period of the study until the sediments were dry. This allowed for easier transportation to a laboratory. Laboratory processing of sediment samples consisted of removing large pieces of plant material and shells and drying the samples for 24 hours in a 60 degree drying oven. Each total sediment sample was weighed (dry weight) and then graded into a standard size fraction series in an automated shaker (Endecotts EFL2000) for 10 minutes at setting 7, using a standard set of test sieves (2000, 1000, 500, 250, 125, 63 µm; Endecotts Ltd). Each fraction was weighed, enabling a size fraction distribution to be calculated. Descriptive terms are those routinely used in sedimentary petrology (Inman 1952): 2000–4000 µm = granule, 1000–2000 µm = very coarse sand, 500 –1000 µm = coarse sand, 250–500 µm = medium sand, 125–250 µm = fine sand, 63–125 µm = very fine sand, sediments less than 63 µm in diameter are referred to generally as silt and clay.

Sediment grain-size was recorded and entered into Microsoft Excel for further analysis. This was then checked for trends and significant differences amongst the treatments.

7. Water quality

Dissolved Oxygen, pH and temperature (°C)

Each quadrat took less than 2 minutes to sample macroinvertebrate abundance and diversity, water nutrients and sediments before water quality was collected. Water quality data was collected for the first five sampling periods leading to the build-up of a bloom. This included dissolved oxygen percentage (D.O.), pH and water temperature (°C). Data was collected each morning for each quadrat every 20 minutes (three times per sampling period). This allowed for a more reliable observation of water quality parameters for each quadrat. Using a Hanna 9145 portable D.O. meter for the dissolved oxygen and water temperature and a Hanna 8424 portable pH/ORP meter for the pH, data were recorded for each quadrat. The probes were

placed inside the cavities left from gathering macroinvertebrate and sediment grain-size samples so interstitial water could be sampled (Figure 11). Interstitial water is more indicative to the habitat of the macroinvertebrates than surface water (Estrella 2013).

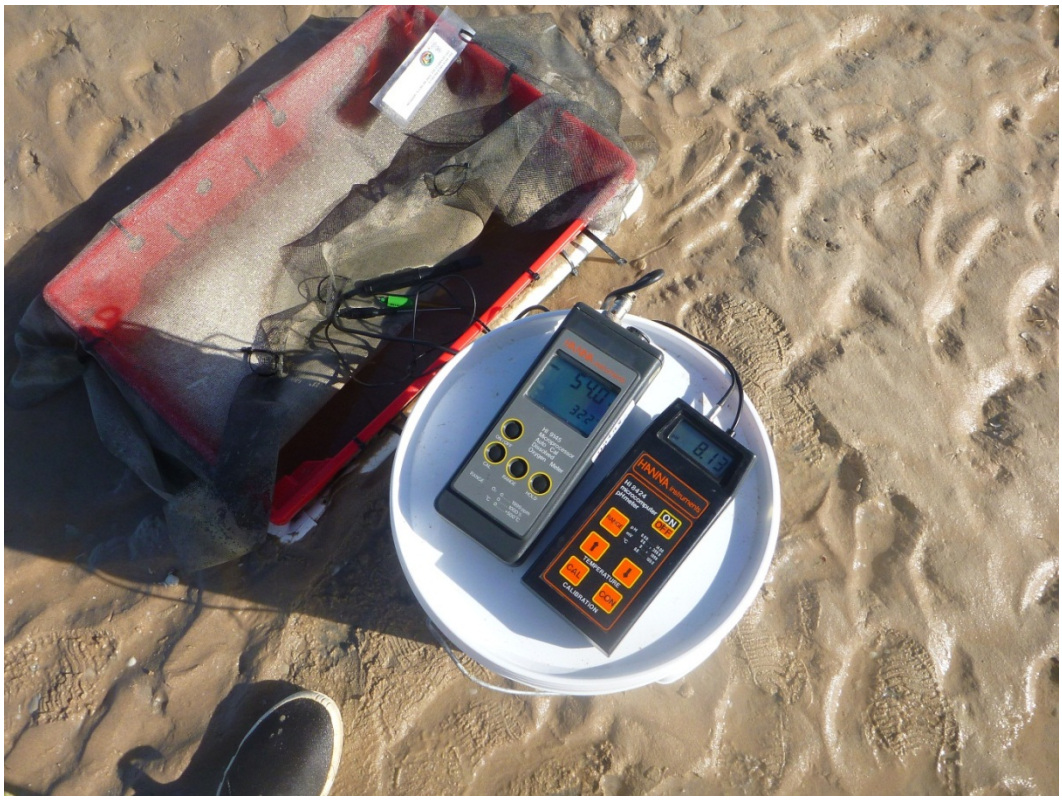

Figure 11. The Hanna 9145 portable DO meter and Hanna 8424 portable pH/ORP meter.

Dissolved Oxygen, pH and temperature (°C) were recorded in all treatment and control quadrats. This data was collected from the first sampling (Day 0) until the fifth sampling (Day 24) every 6 days as this encompassed the largest changes over the bloom (Ahern *et al.* 2008).

Water nutrients- nitrates and phosphates

Nitrate and phosphate of seawater were collected on days 0, 6, 12, 18 and 24 to test for nutrients. Samples were taken directly after the macroinvertebrate sampling at low tide by using a Terumo 60 mL syringe and filling up a 500 mL container with the sample. This was then cooled to – 4 ° C for 24 hours before extracting the water and filtering through a 0.45 micron filter (Figure 12). The sample nutrient content was analysed with Odyssey Hach powder pillows and an Odyssey DR/2010 Spectrophotometer. Nitrates were measured using the Cadmium reduction method with NitraVer® Nitrate Reagent and phosphates using the PhosVer® 3 Ascorbic acid method with PhosVer (Hach 1999; Ng *et al.* 2012).

Figure 12. Terumo 60 mL syringe with 0.45 micron filter attached.

8. Statistical methods.

The collected raw data from each of the variables was plotted on graphs and charts to compare trends over time using Microsoft Excel (2010). Descriptive statistics (mean, standard error, standard deviation) were calculated to provide a summary of the data. The Shannon-Weiner diversity index was utilised for relative richness of the macroinvertebrate assemblage using the species richness recorded and abundance for each sample and each treatment, evenness or Shannon's equitability constant was then calculated. Differences between the macroinvertebrate diversity and abundance between sampling dates and treatments were quantified through univariate statistics using the IBM SPSS Statistics for Windows, Version 21.0. Data was tested for normality using the Shapiro-Wilk test.

To test for temporal differences within treatments a repeated measure one-way analysis of variance (ANOVA) with sampling dates as a fixed factor was carried out for abundance and species richness, sediment grain size and water quality. Where the requirements were not met for a repeated measure ANOVA, a standard one-way analysis of variance (ANOVA) was undertaken with Scheffe post hoc adjustments. Species rank and natural log tables were

calculated using Microsoft Excel to show which species had the highest abundance in each treatment in descending order.

Independent sample t-tests were performed to test for differences between fixed quadrat vs. random quadrat, control vs. impact, sediment grain-size, analysis, water quality variables (D.O., pH and Temperature) and water nutrients (phosphorus and nitrogen).

Results

1. Macroinvertebrate Abundance and Diversity

Nine assessments in 3 replicate quadrats in a control (*Lyngbya majuscula* absent) and impact (*Lyngbya majuscula* present) were made over 53 days (28/02/2013 – 22/04/2013) every 6 days where possible. The following variables were assessed for each sample period: macroinvertebrate total abundance and total species richness. Shannon's diversity index and evenness, % of relative abundance (species distribution of the macroinvertebrate assemblage) were subsequently calculated.

Quadrat effects on macroinvertebrate abundance and diversity

This study was designed to show the effects of a gradual *Lyngbya majuscula* bloom on macroinvertebrates in an enclosed environment with frequent sampling dates. This required the use of box quadrats placed permanently in the environment for the duration of the study. To make sure the quadrats were not a factor, a treatment of random unfixed controls were included to test against the fixed control quadrats (See Study design). The results of this were interpreted as significant at the $p = 0.05$ level. Impact fixed quadrats could not be tested against, as *Lyngbya* had been added and were hypothesised to be different from the control. This was carried out by comparing abundance and richness data between the two types of control for the duration of the experiment (53 days). Data was confirmed to be normal between treatments using the Shipiro-Wilk test.

Macroinvertebrate abundance of fixed and random quadrats is summarised in (Figure 13). No significant difference was found between the macroinvertebrate abundance of fixed and random quadrats ($t_{(52)} = 1.518$, $p = 0.135$). The inclusion of the fixed quadrat to allow for in situ treatment did not have a significant effect on abundance.

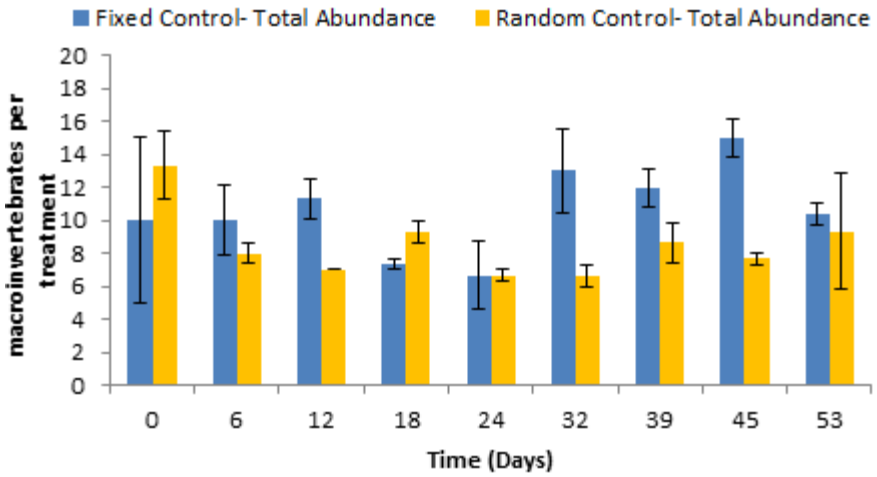

Figure 13. Mean abundance of macroinvertebrates in random control and fixed control quadrats with (+/- SE) February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

Macroinvertebrate species richness of fixed and random quadrats is summarised in (Figure 14). No significant difference was found between the macroinvertebrate species richness of fixed and random quadrats ($t_{(52)} = 1.218$, $p = 0.229$). The inclusion of the fixed quadrat to allow for in situ treatment did not have a significant effect on species richness.

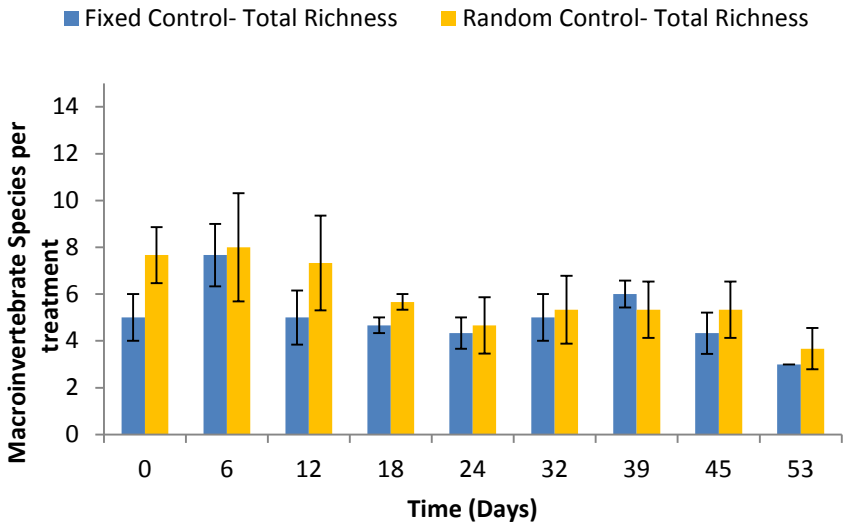

Figure 14. Mean species richness in random control and fixed control quadrats (+/- SE) February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

These results suggest that the fixed nature of the impact quadrats did not significantly affect macroinvertebrate abundance or species richness. The fixed control could to be used against the fixed impact without significant disturbance from the enclosed quadrat structure.

2. Treatment effect on macroinvertebrate abundance

Total abundance of macroinvertebrates recorded in the fixed control and fixed impact through nine samplings was 517 individuals (287 control; 230 impact), representing 39 taxa (Table 3). Within the controls the abundance ranged from 22 individuals on day 24 to 45 individuals on day 53 (Table 3). Within the impacts the amount of abundance ranged from 20 individuals on day 45 to 40 individuals on day 0 (the first sample date) (Table 3). Mean abundance of each macroinvertebrate was calculated in the laboratory for each sampling period for total individuals per sampling period (Figure 15).

Table 3. Summary of sampling dates, control total abundance and richness and impact total abundance and richness 28/02/2013- 22/04/2013.

Sample Date	Day	Control Total Abundance	Control Species Richness	Impact Total Abundance	Impact Species Richness
February 28, 2013	0	30	10	40	16
March 6, 2013	6	30	16	24	9
March 12, 2013	12	34	10	21	7
March 18, 2013	18	22	9	28	11
March 24, 2013	24	20	9	20	10
April 2, 2013	32	39	10	20	8
April 8, 2013	39	36	11	26	8
April 14, 2013	45	45	9	23	6
April 22, 2013	53	31	4	28	4
TOTALS		287	29	230	29

Macroinvertebrate abundance of control and impact treatments is summarised in Figure 15.The lowest macroinvertebrate mean abundance was in day 24 in both control and impact treatments. Mean abundance was highest in the control quadrats during day 45. For the impact mean abundance was highest during day 0 or the first sampling date.

A significant difference was found between the macroinvertebrate abundance of control and impact treatments ($t_{(52)} = 2.192$, $p = 0.033$).

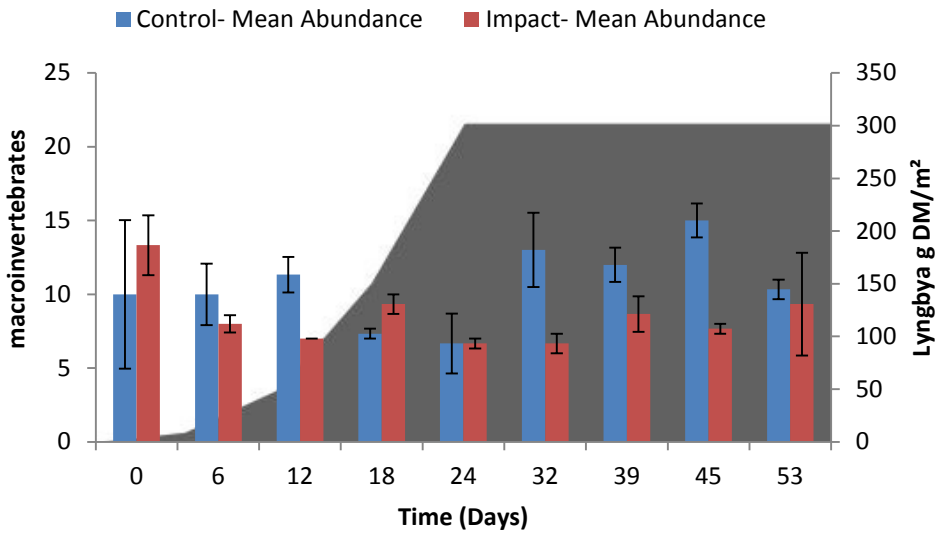

Figure 15. Mean abundance of macroinvertebrates in control and impact (+/- SE), February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

Sampling dates were used as a factor in the fixed control and impact quadrats to check whether there were natural trends of abundance over time at the study site. A repeated measure ANOVA with a Greenhouse-Geisser correction determined that mean abundance of the fixed control showed no significant difference, $F(8, 21) = 1.056$, $p = 0.428$. Post hoc tests using the Bonferroni correction revealed no significant difference between time points. The random controls were also tested to account for natural variation under the same corrections and found no significant difference, $F(8, 27) = 2.140$, $p = 0.067$. Post hoc tests using the Bonferroni correction revealed no significant difference between time points.

A repeated measure ANOVA with a Greenhouse-Geisser correction determined that mean abundance of the impacted quadrats had significant differences between sampling occasions at the 0.05 level, $F(8, 21) = 2.576$, $p= 0.039$. Post hoc tests using the Bonferroni correction revealed statistically significant differences from day 0, day 12, day 24, day 32 and day 45. Overall abundance increases in the control but did not increase in the impact.

The most abundant of the macroinvertebrates recorded over the whole study were the foraminifera, in both control (149 individuals) and impact (118 individuals). Foraminifera had been found in every sampling period for each treatment. Foraminifera increased in both control and impact over the period of the study (Figure 16). No significant difference was found between the foraminifera abundance of control and impact treatments (t-test: $t_{(52)} = 1.917$, $p = 0.185$).

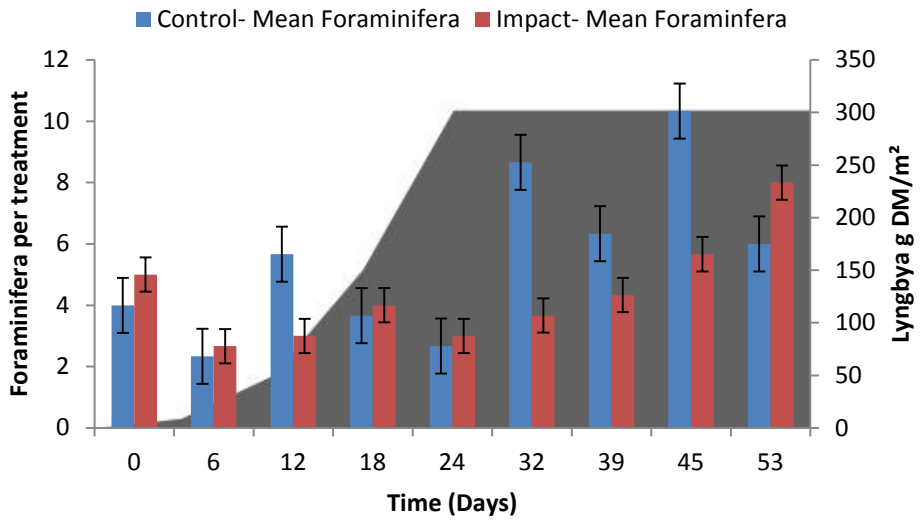

Figure 16. Mean number of foraminifera recorded in each sampling period, February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

Second in abundance was the polychaete worm, Spionidae, and was recorded 26 times in the control quadrats (Table 4) and 26 times in the impact quadrats (Table 5) throughout the experiment (Figure 17). No significant difference was found between the Spionidae abundance of control and impact treatments (t-test: $t_{(52)} = 0.13$, $p = 0.909$).

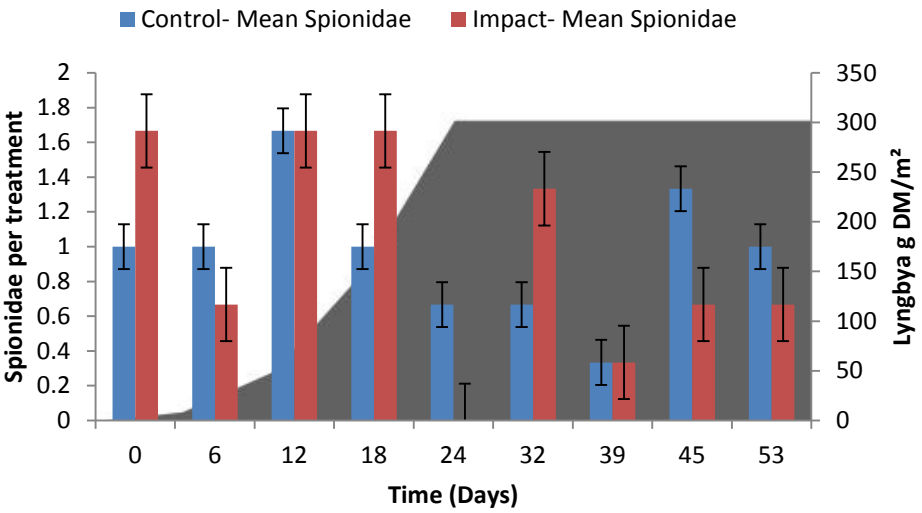

Figure 17. Mean number of spionidae recorded in each sampling period, February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

For the control quadrats, third in abundance was an unidentifiable polychaete worm (15, n=9) and fourth in abundance was the polychaete worm, Paraonidae (15, n=9) (Table 4). Third in abundance for the impact quadrats was gastropod species 1 (17, n=9) and fourth was the unidentifiable polychaete worm (9, n=9) (Table 5). For both the control quadrats and the

impact quadrats, ten species were found only once over the period of the experiment (Table 3). Polychaete worms ranked highly in both quadrat types and were pooled together for trends (Figure 18). No significant differences were found between the polychaete abundance of control and impact treatments (t-test: $t_{(52)} = 0.234$, $p = 0.234$). Polychaetes in general increased in the control and declined in the impact.

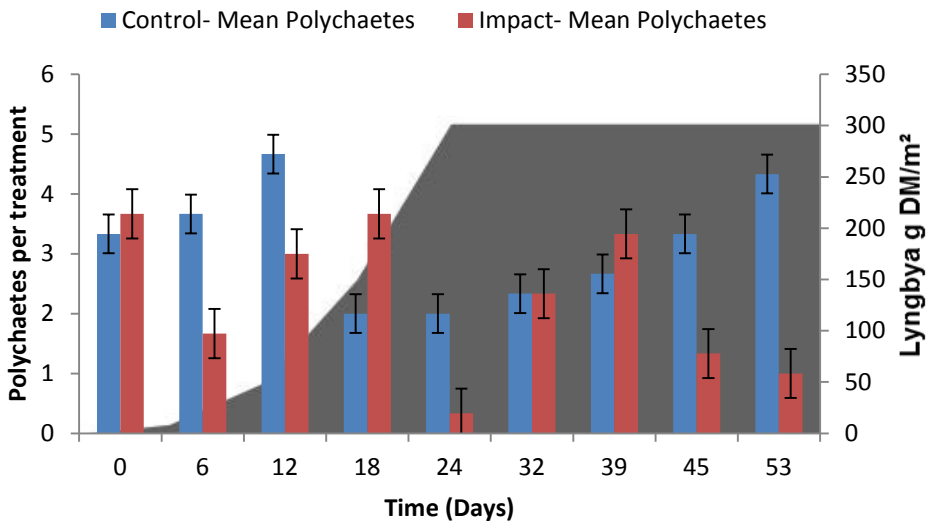

Figure 18. Mean number of polychaetes recorded in each sampling period, February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

Gastropod species 1 did not have large enough numbers to test for trends. Therefore, all gastropods were pooled together as gastropods ranked highly in both control (Table 4) and impact (Table 5). The overall gastropod abundance is summarised in Figure 19. No significant differences were found between the gastropod abundance of control and impact treatments (t-test: $t_{(52)} = -0.714$, $p = 0.486$). However, overall gastropods in both control and impact significantly decreased from day 0 to day 53 (Figure 19).

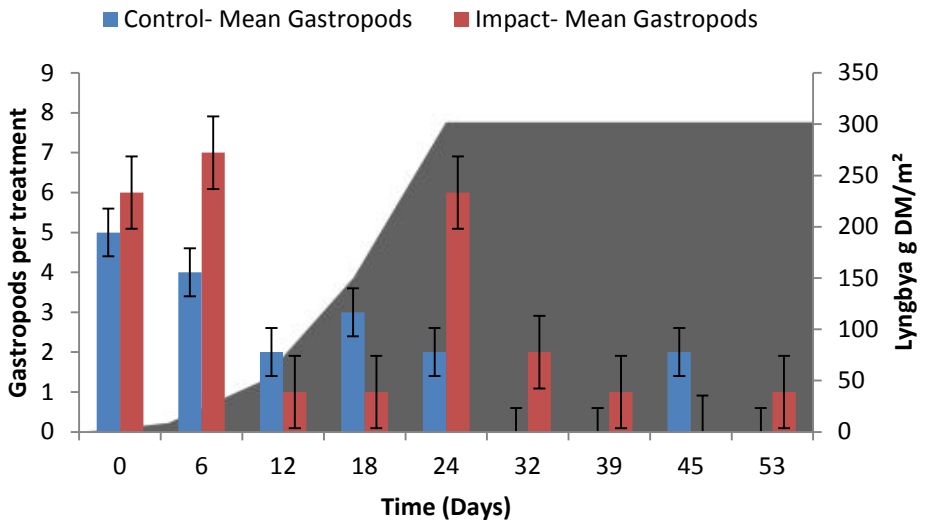

Figure 19. Mean number of gastropods recorded in each sampling period, February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

Bivalves ranked in the top ten for both control and impact quadrats (Table 4 and Table 5) and were pooled together for trends (Figure 20). No significant differences were found between the bivalve abundance of control and impact treatments (t-test: $t_{(52)} = 0.494$, $p = 0.628$).

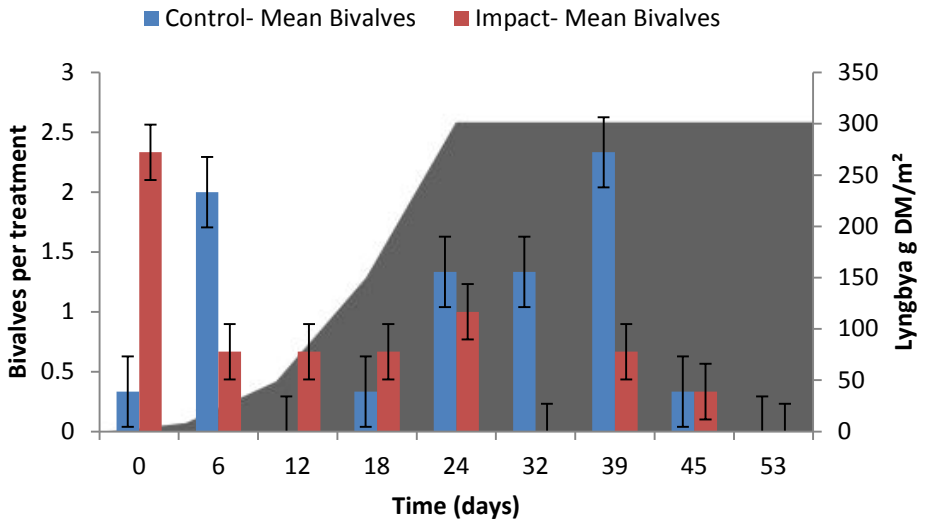

Figure 20. Mean number of bivalves recorded in each sampling period, February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

3. Treatment effects on Species Richness

Control quadrats had 10 unique taxa not found in the impact quadrats. These species were Antheluridae, Cumacea, Gammaridea, Macrothalmus spp., Magelonidae, Megapoda spp., Ostracoda sp. 2, Ostracoda sp. 3, *Placomen callophylum* and *Solemya cf terrareginae* (Table 4).

Table 4. Rank and log abundance of all control quadrats recorded 28/02/2013- 22/04/2013.

Rank	Genus Species	Log	Rank	Genus Species	Log
1	Foraminifera	5.00	16	Heterocardia gibbosula	0.69
2	Spionidae sp.	3.26	17	Laevidentalium cf lubricatum	0.69
3	Polychaete (damaged)	2.71	18	Antheluridae	0.69
4	Paraonidae sp.	2.71	19	Magelonidae sp.	0.69
5	Nereidae sp.	2.56	20	Amphiura tenuis	0.00
6	Gastropoda sp. 1	2.40	21	Mactra grandis	0.00
7	Tellina capsoides	2.20	22	Tellina piratica	0.00
8	Gastropoda sp. 2	1.95	23	Cumacea spp.	0.00
9	Nephtyidae sp.	1.61	24	Gammaridae	0.00
10	Solemya cf terraereginae	1.61	25	Macrophthalmus spp.	0.00
11	Oweniidae sp.	1.39	26	Megapoda	0.00
12	Bivalve (unidentifiable)	1.10	27	Ostracoda sp.2	0.00
13	Syllidae sp.	1.10	28	Ostracoda sp.3	0.00
14	Anomalocardia squamosa	0.69	29	Placomen callophylum	0.00
15	Glyceridae sp.	0.69			

Impact quadrats had 10 unique taxa not found in the control quadrats. These species were *Anadara granosa*, Caridea sp., Cirratulidae sp., Epitoniidae sp., Gastropod (damaged), Neritidae sp., Ostracoda sp. 4, Pantapoda sp., *Tellina exotica* and *Tellina oval* (Table 5).

Table 5. Rank and log abundance of all impact quadrats recorded 28/02/2013- 22/04/2013.

Rank	Genus Species	Log	Rank	Genus Species	Log
1	Foraminifera	4.77	16	Laevidentalium cf lubricatum	0.69
2	Spionidae sp.	3.26	17	Anadara granosa	0.69
3	Gastropoda sp. 1	2.83	18	Epitoniidae spp.	0.69
4	Polychaete (damaged)	2.20	19	Ostracoda sp.4	0.69
5	Nereidae sp.	2.08	20	Amphiura tenuis	0.00
6	Tellina capsoides	1.61	21	Mactra grandis	0.00
7	Nephtyidae sp.	1.61	22	Tellina piratica	0.00
8	Paraonidae sp.	1.39	23	Caridea spp.	0.00
9	Gastropoda sp. 2	1.39	24	Cirratulidae sp.	0.00
10	Anomalocardia squamosa	1.39	25	Gastropod(unidentifiable)	0.00
11	Glyceridae sp.	1.39	26	Neritidae spp.	0.00
12	Oweniidae sp.	0.69	27	Pantopoda spp.	0.00
13	Bivalve (unidentifiable)	0.69	28	Tellina exotica	0.00
14	Syllidae sp.	0.69	29	Tellina oval	0.00
15	Heterocardia gibbosula	0.69			

Some of these unique species were found to have high rankings whilst most were only found a few times over the nine sampling dates (Table 6). The highest ranking control only species were *Solemya cf terrareginae* (5, n=9), antheluridae (2, n=9) and magelonidae (2, n=9) at rank 10, 17 and 18 respectively. The highest ranking impact only species were *Anadara granosa* (2, n=9), Epitoniidae spp. (2, n=9) and ostracod sp. 4(2, n=9) at rank 17, 18 and 19 respectively.

Table 6. Species only found in each treatment (Control Vs. Impact) with rankings and log 28/02/2013- 22/04/2013.

Species only found in the control quadrats			Species only found in the impact quadrats		
Species Rank in control	Species	Log	Species Rank in impact	Species	Log
10	Solemya cf terraereginae	1.61	17	Anadara granosa	0.69
18	Antheluridae	0.69	18	Epitoniidae spp.	0.69
19	Magelonidae sp.	0.69	19	Ostracoda sp.4	0.69
23	Cumacea spp.	0.00	23	Caridea spp.	0.00
24	Gammaridae	0.00	24	Cirratulidae sp.	0.00
25	Macrophthalmus spp.	0.00	25	Gastropod (unident.)	0.00
26	Megapoda	0.00	26	Neritidae spp.	0.00
27	Ostracoda sp.2	0.00	27	Pantopoda spp.	0.00
28	Ostracoda sp.3	0.00	28	Tellina exotica	0.00
29	Placomen callophylum	0.00	29	Tellina oval	0.00

Overall macroinvertebrate species richness of control and impact quadrats is summarised in (Figure 21). No significant difference was found between the macroinvertebrate species richness of control and impact quadrats (t-test: $t_{(52)} = 1.536$, $p = 0.131$).

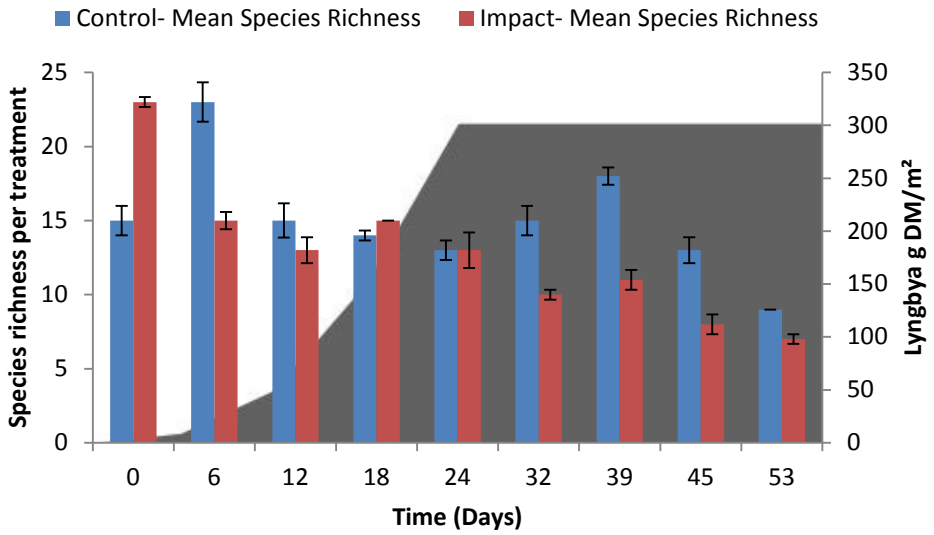

Figure 21. Mean Species Richness of macroinvertebrates in control and impact (+/- SE), February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m²).

A repeated measure ANOVA with a Greenhouse-Geisser correction determined that mean species richness of the control quadrats had significant differences, $F(8, 21) = 4.930$, $p = 0.002$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 0 and day 6, 18, 24, 45, and 53. This showed an overall decrease in species richness. There were also significant differences between day 53 and day 12, 32, 39, and 45. The Random control quadrats were tested to account for natural variation using the same corrections and found to be significantly different $F(8, 27) = 2.422$, $p = 0.041$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 0 and day 53, and between day 6 and day 18, 24, and 53. This also showed a decline in species richness.

A repeated measure ANOVA with a Greenhouse-Geisser correction determined that mean species richness of the impact quadrats had significant differences, $F(8, 21) = 4.055$, $p = 0.005$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 0 and day 12, 24, 32, 45, and 53, and between day 53 and day 18. The overall species richness declined steadily in the impact treatment from day 18 onwards (Figure 21).

4. Diversity Indices

Greatest Shannon diversity, ($H^1 = 2.514$) occurred in the control treatment on day 6 (Figure 22) and coincided with the highest evenness, ($Eh = 0.907$) (Figure 23). This sampling period had the lowest abundance to species richness. In the impact the greatest Shannon diversity, ($H^1 = 2.202$) occurred on day 0 and had the third highest evenness of all the sampling dates. The highest evenness in the impact was on day 6 with an $Eh = 0.848$.

The lowest of each measure also occurred in the same sampling period, day 53 with control ($H^1 = 1.068$) and impact ($H^1 = 0.559$) in Shannon's diversity index. No significant difference were found between the control and impact quadrats in the Shannon diversity index (t-test: $t_{(52)} = 0.684$, $p = 0.504$). This is similar to the results of the species richness (Figure 21). Evenness was also not significantly different between the impact and control (t-test: $t_{(52)} = 0.632$, $p = 0.674$).

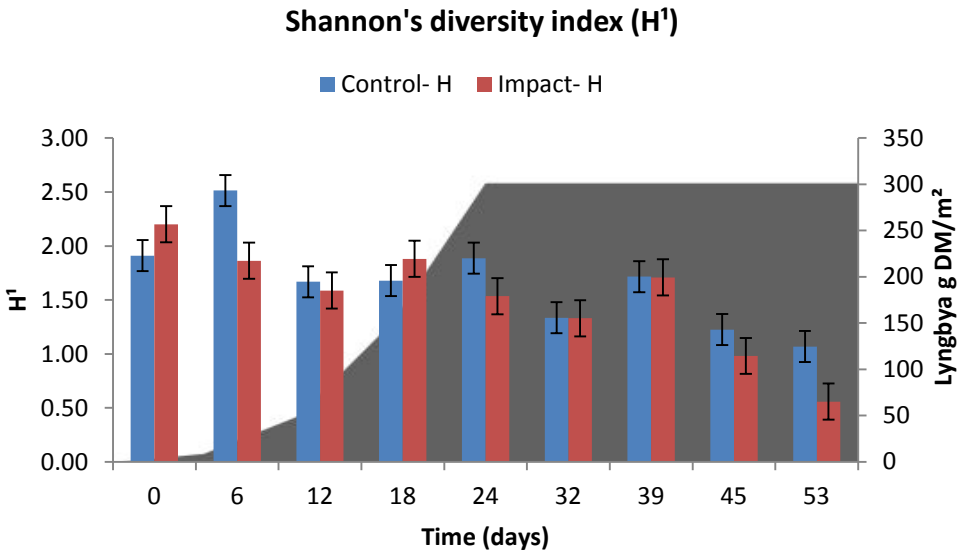

Figure 22. Shannon's diversity index (H^1) between control and impact (+/- SE), February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m^2).

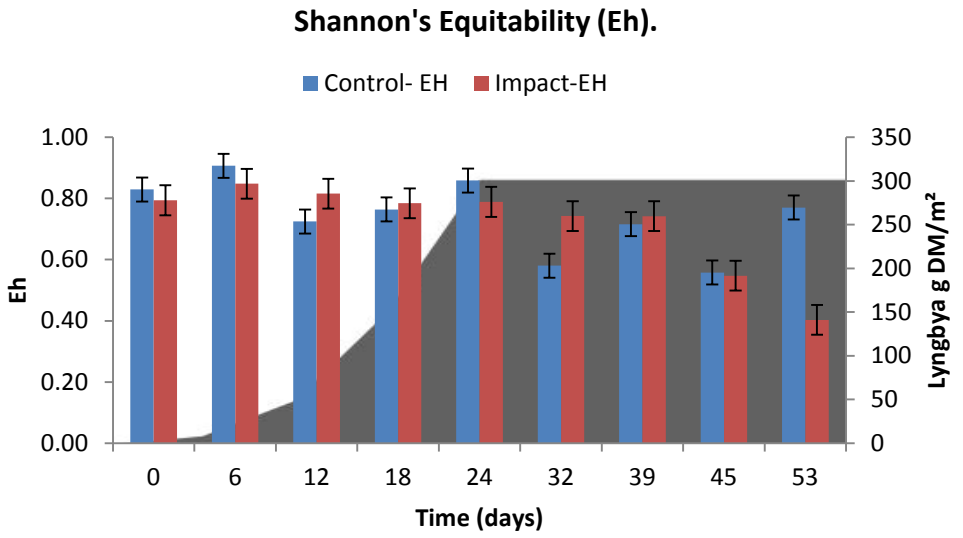

Figure 23. Shannon's Equitability (Eh) between control and impact (+/- SE), February 28, 2013- April 22, 2013 with Lyngbya biomass shadowed in back ground (0-300g DM/m^2).

5. Sediment grain size

Examination of the sediment size fraction characteristics reveals a slightly higher proportion of very fine sand (< 0.25 mm) in the controls than the impacts (Figure 24).

A repeated measure ANOVA with a Greenhouse-Geisser correction found significant differences in the control quadrats with coarse sand (≤ 0.50 mm), $F(8, 26) = 2.751$, $p = 0.036$ and very fine sand (≤ 0.63 mm), $F(8, 26) = 2.536$, $p = 0.048$ size fractions. Post hoc tests using the Bonferroni correction revealed no statistically significant differences between time points. The very fine sediment increased overtime while the coarse sediment decreased overtime in the control quadrats.

A repeated measure ANOVA with a Greenhouse-Geisser correction found significant differences in the impact quadrats with coarse sand (≤ 0.50 mm), $F(8, 26) = 2.777$, $p = 0.034$, medium sand (≤ 0.25 mm) $F(8, 26) = 3.434$, $p = 0.014$, and fine sand (≤ 0.125 mm), $F(8, 26) = 3.215$, $p = 0.019$. Post hoc tests using the Bonferroni correction revealed no statistically significant differences between time points. Very fine sand (> 0.63 mm), was also found to be significantly different, $F(8, 26) = 5.555$, $p = 0.001$ and post hoc tests using the Bonferroni correction show significant differences between day 6 and day 12. Coarse and medium sand increased, while fine sand decreased over the period of the study within the impact quadrats.

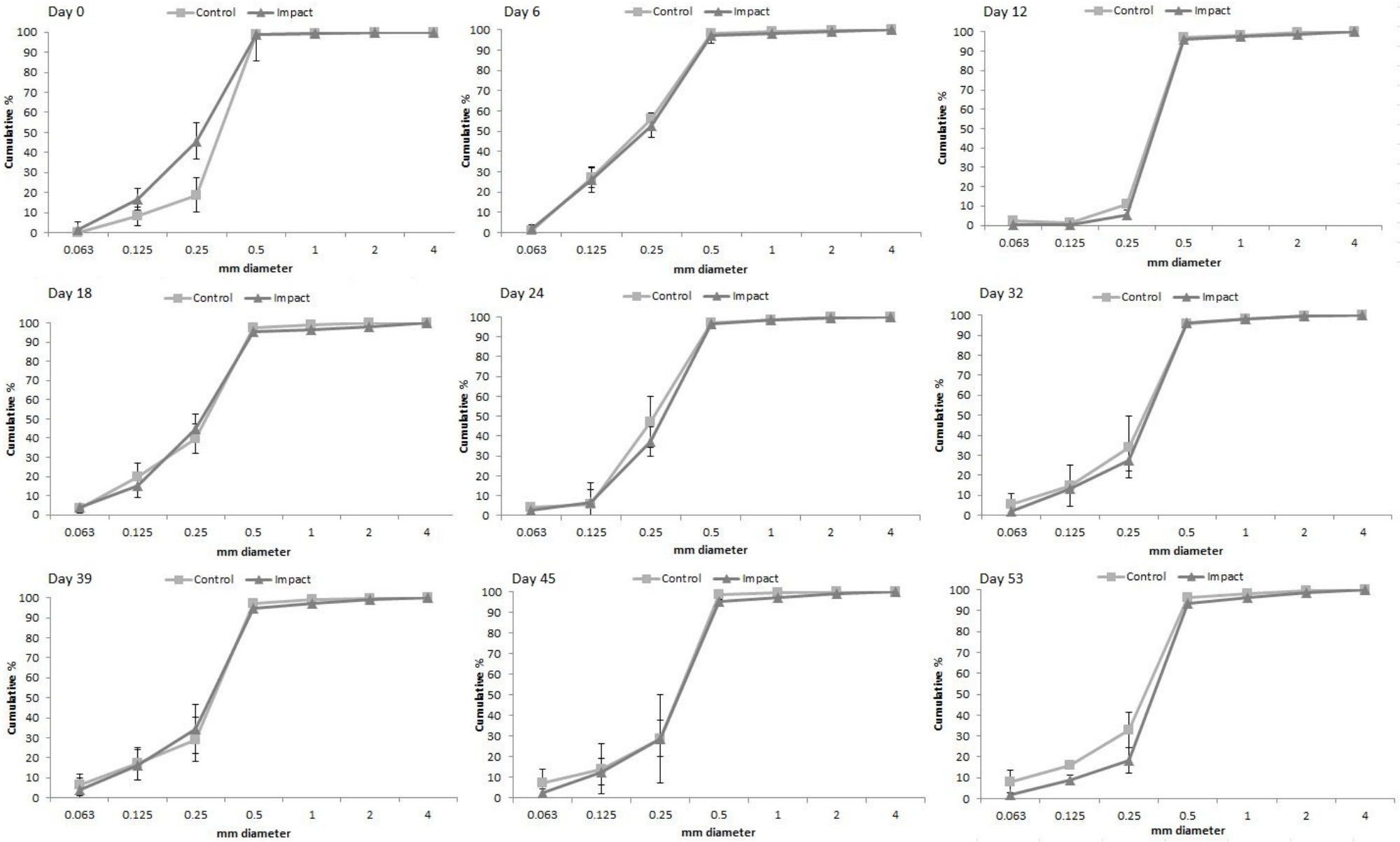

Figure 24. Cumulative % of grainsize for each sampling day between control and impact (+/- SE) February 28, 2013- April 22, 2013.

6. Water Quality

Dissolved Oxygen, pH and temperature (°C)

Dissolved oxygen percent saturation (D.O. %) and pH were tested against the random controls to check whether the fixed quadrats were a factor. D.O. % ($t_{(28)} = -2.182$, $p = 0.061$) and pH ($t_{(28)} = -0.670$, $p = 0.522$) both showed no significant differences when testing if fixed quadrats affected these parameters. Temperature was not used as a variable between treatments.

For D.O. %, the highest mean was recorded on day 24 in the impact quadrats at (106.24 ± 15.95 % O_2). The lowest mean D.O. % was recorded in the control quadrats during day 6 of the experiment (72.76 ± 1.93 % O_2). D.O. % of control and impact quadrats is summarised in (Figure 25). A significant difference was found between the control and impact quadrats ($t_{(28)} = -2.192$, $p = 0.039$).

A repeated measure ANOVA with a Greenhouse-Geisser correction determined that mean D.O. % of the control quadrats had significant differences, $F(1, 8) = 11.982$, $p = 0.009$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 0 and day 24; day 6 and day 24; day 18 and day 24. This showed an overall increase in D.O. %. The Random control quadrats were tested to account for natural variation under the same corrections and found to be significantly different, $F(1, 8) = 8.314$, $p = 0.020$. Post hoc tests using the Bonferroni correction revealed that all the sampling dates except day 18 had significant differences with other days.

The impact quadrats were also found to be significant, $F(1, 8) = 40.987$, $p = 0.001$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 24 and all the other time points. From day 12 the D.O. % in the impact quadrats increases greatly (Figure 25).

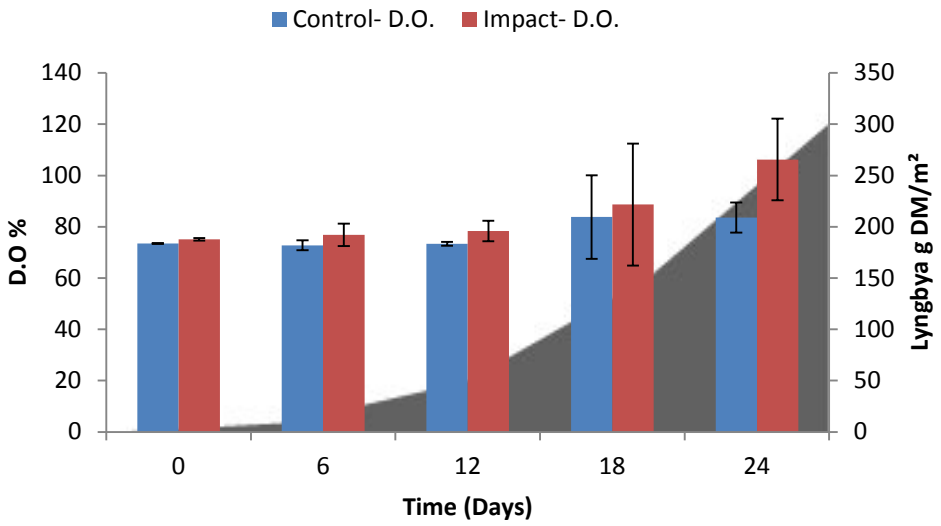

Figure 25. Mean dissolved oxygen percentage across quadrats (SE +/-) between control and impact over five sampling periods with increasing Lyngbya biomass shaded in background (0-300g DM/m²).

For pH values, the highest mean recorded was in the control quadrats on day 24 of the study (8.531, n = 5) and the lowest was found in the impact quadrats during the first sampling (day 0) in the impact quadrats (8.223, n = 5). pH of control and impact quadrats is summarised in (Figure 26). No significant difference was found between the control and impact quadrats ($t_{(28)} = 1.977$, $p = 0.058$).

A repeated measure ANOVA with a Greenhouse-Geisser correction determined that mean pH of the control quadrats had significant differences, $F(1, 8) = 8.086$, $p = 0.021$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 0 and all other days; and day 24 with day 6. This showed an overall increase in pH (Figure 26). The Random control quadrats were tested to account for natural variation using the same corrections and found to be significantly different, $F(1, 8) = 7.892$, $p = 0.022$. Post hoc tests using the Bonferroni correction revealed that all day 0 was significantly different to all other days. There was an overall increase in the pH of the random control quadrats.

The impact quadrats were also found to be significant, $F(1, 8) = 8.086$, $p = 0.021$. Post hoc tests using the Bonferroni correction revealed statistically significant differences between day 0 and all the other time points; and day 6 with day 24. This showed an overall increase similar to the control quadrat but at lower levels (Figure 26).

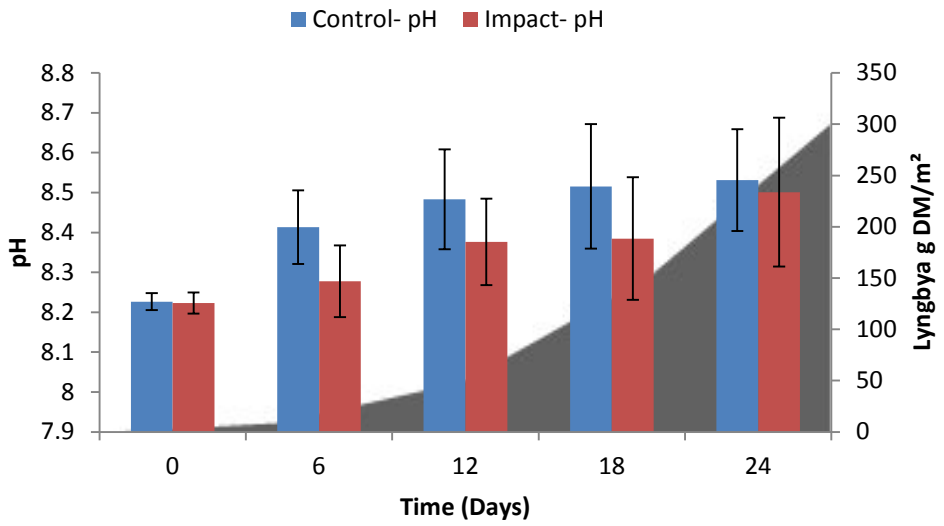

Figure 26. Mean pH across quadrats (SE +/-) between control and impact over five sampling periods with increasing Lyngbya biomass shaded in background (0-300g DM/m²).

Water nutrients- nitrates and phosphates

Nitrogen and phosphorus demonstrated some differences between control and impact. The effects of the quadrats as a factor were tested with significant differences found between the random control and the control in the nitrates ($t_{(8)} = -3.910$, $p = 0.004$) but not the phosphates ($t_{(8)} = -1.924$, $p = 0.091$). Nitrates had the highest mean value in the control quadrats (8.213, n = 5) during day18 and the lowest in the impact (3.96, n = 5) during day 6 of the study. Nitrate levels of control and impact quadrats is summarised in (Figure 27). No significant difference was found between the control and impact quadrats ($t_{(28)} = 1.652$, $p = 0.110$).

Sampling dates were used as a factor in the fixed control and impact quadrats to check whether there were natural trends over time at the study site. Significant differences in nitrate mg L^{-1} were found in the impact, $F(4, 14) = 7.174$, $p = 0.005$ but not in the control $F(4, 14) = 1.281$, $p = 0.340$. Scheffe adjustments showed significant differences in between day 6 and day 12, and day 18. *Lyngbya* biomass increased from 10 DM/m² to 50 DM/m² and then 150 DM/m² in these sampling periods

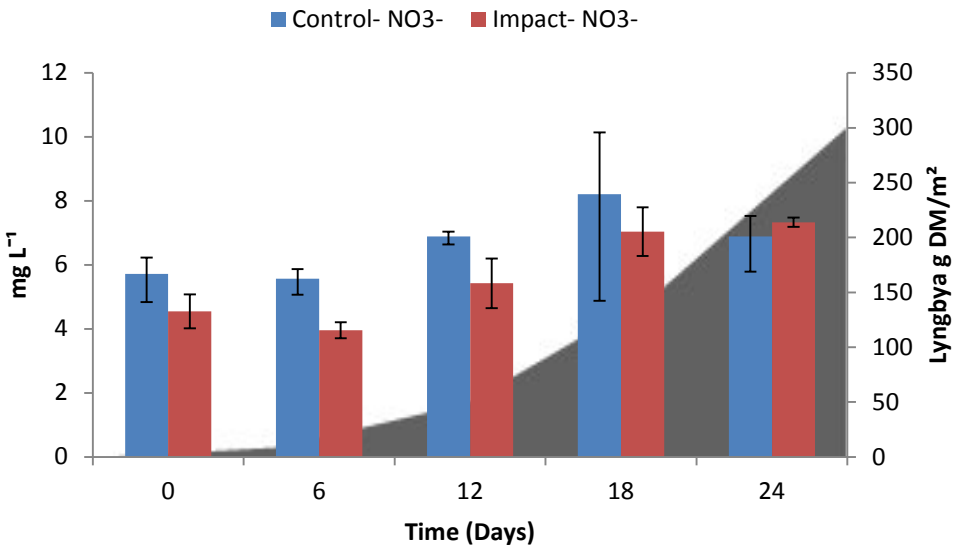

Figure 27. Mean nitrates across quadrats (SE +/-) between control and impact over five sampling periods with increasing Lyngbya biomass shaded in background (0-300g DM/m²).

Phosphates had the highest mean value on day 18 in the impact quadrats (6.026, n= 5) and the lowest also in the impact quadrats (0.48, n= 5) on day 24 of the study. Phosphate levels of control and impact quadrats is summarised in (Figure 28). No significant difference was found between the control and impact quadrats ($t_{(28)} = 0.805$, $p = 0.428$).

Sampling dates were used as a factor in the fixed control and impact quadrats to check whether there were natural trends over time at the study site. Significant differences were found in the impact, $F(4, 14) = 6.916$, $p = 0.006$. A Scheffe adjustment showed between the day 18 and day 24 there were significant differences (Figure 28). No significant difference in were found in the control, $F(4, 14) = 2.279$, $p = 0.133$.

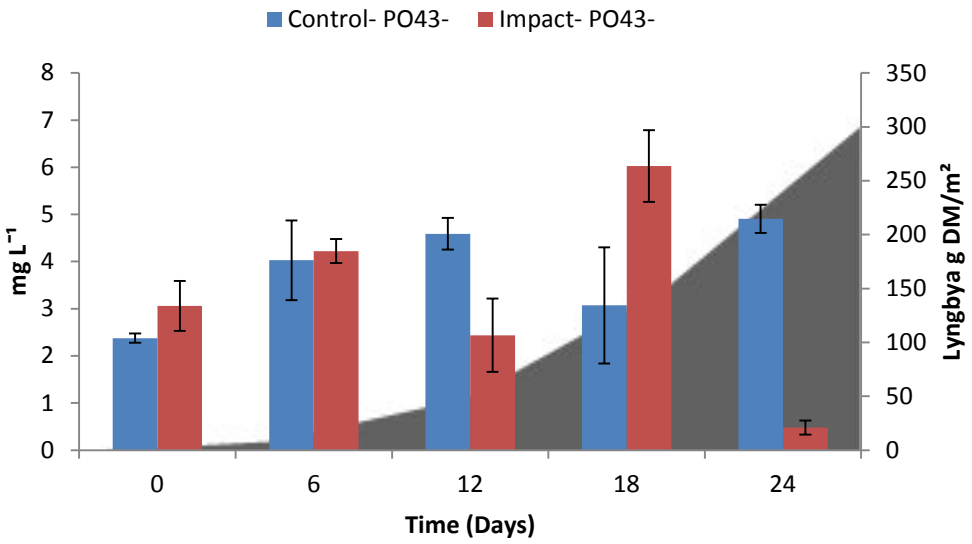

Figure 28. Mean phosphates across quadrats (SE +/-) between control and impact over five sampling periods with increasing Lyngbya biomass shaded in background (0-300g DM/m²).

Discussion

Macroinvertebrates

The results from two months of frequent sampling supported the hypothesis that *Lyngbya majuscula* does negatively alter macroinvertebrate diversity as other studies have pointed out (Dennison *et al.* 1999; Garcia and Johnstone 2006; Estrella *et al.* 2011; Estrella 2013; Paerl and Otten 2013). Some specific macroinvertebrate taxa were found to be more effected than others, for example: foraminifera, polychaetes, bivalves and gastropods. This is either because of opportunistic species which thrive in these conditions, such as the sipunculid worm (Estrella 2013), grazing gastropods like *Bulla* sp. or *Hamineoidae* sp. (Estrella *et al.* 2011), or sea hares (Capper *et al.* 2005; Geange and Stier 2010), that can change the food chain of an ecosystem (Geange and Stier 2010). Some of the changes in abundance and species richness could also be attributed to natural seasonal change found in these ecosystems (Metcalfe and Glasby 2008).

On the other hand, Foraminifera abundance increased similarly in both the impact and the control, suggesting they are not significantly affected by the presence of *Lyngbya*. This could be due to available nutrients found within the treatments and the potential symbiotic relationship with algae many of the marine species have (Uthicke and Nobes 2008; Alve and Goldstein 2010).

Another possible reason for such an increase could be due to the short life cycles and multiple rounds of asexual reproduction commonly found in marine foraminifera (Nobes *et al.* 2008; Alve and Goldstein 2010).

In general, polychaetes were found to be declining in the presence of *Lyngbya* but increasing in the control from day 18. This goes against previous studies where polychaetes tend to increase in the presence of *Lyngbya majuscula* during February-March in Roebuck Bay (Estrella 2013). Results of the current study are more in line with similar studies in Queensland, where polychaete abundance decreased with a bloom of *Lyngbya majuscula* (Garcia and Johnstone 2006). The formation of a natural *Lyngbya majuscula* bloom did not occur in this study and may be responsible for the lack of potential prey that would benefit from a bloom for polychaetes to feed on (Estrella *et al.* 2011). Polychaetes such as Spionidae and Paraonidae are surface deposit feeders and could benefit from wide-ranging higher biomass created by the formation of natural bloom (Day 1967; Fauchald and Jumars 1979). The artificial bloom this study propagated may not have met all the requirements for these ecological processes to eventuate (Ahern *et al.* 2007).

Spionidae was found to decrease in the presence of *Lyngbya* (impact) whilst staying relatively stable in the control. The inability to migrate away from *Lyngbya majuscula* during hypoxic/anoxic conditions may have caused this decrease in numbers but further studies would be required (Wear and Gardner 2001). Paraonidae showed a more pronounced decrease in the impact treatment but increased a great deal in the controls from Day 32 until Day 53. This may be due to being less tolerant than spionid species which can survive in short term hypoxic (low tide) conditions relatively well (Daunys *et al.* 2000; Glasby *et al.* 2000). The other polycheates of note, Nereidae and Nephtyidae both showed sporadic accounts of abundance and further study would be required to understand this (O'Brien *et al.* 2010). Polychaetes in general are more resistant to changes in environment than many other macroinvertebrates (Glasby *et al.* 2000).

Gastropods showed a decline in all species. This was however different from previous studies where gastropods increased in abundance in the presence of high *Lyngbya majuscula* densities (Estrella 2013). Gastropods are usually very tolerant of bloom events (Langenbuch and PÖrtner 2004; Vaquer-Sunyer and Duarte 2008).

The gastropod *Haminoeidae* was found in high numbers in previous bloom events in this area but none were found in either the control or impact and this may be due to conditions that did not support a naturally occurring bloom (Hamilton *et al.* 2009; Johnson *et al.* 2010).

Bivalves showed a large decrease in the impacts, especially after bloom biomass has been achieved. The controls on the other hand show an increase during this period. This was a different result to what was seen in other studies (O'Brien *et al.* 2010; Estrella *et al.* 2011). The reasoning could be as simple as the reproduction cycle of the bivalves involving spawning cannot pass through the mesh of the quadrats due to being held in by the *Lyngbya majuscula*.

Ostracods, amphipods and isopods did not contribute much to the whole macroinvertebrate assemblage but all declined to zero by the end of the study in both control and impact treatments. Amphipods and isopods have been noted to have low tolerance to anoxic/hypoxic environments which could explain their disappearance in the impact treatments (Garcia and Johnstone 2006). This could be due to possible reproduction cycle issues with the mesh of the quadrats or it could just be a seasonal occurrence in abundance for these taxa (Estrella *et al.* 2011).

Species richness supports previous studies where richness overall declines in the presence of *Lyngbya majuscula* (Estrella *et al.* 2011; Estrella 2013). This suggests that significant changes occurred after the bloom biomass (300 g DM/m^2) had been reached (Day 24) when compared to the first sampling, day 0.

Sediments

Sediments overall did not show many significant differences over the period of the study. This supports other studies during a natural *Lyngbya majuscula* bloom (Garcia-Novoa 2003). However, for the grain size differences that were significant, a reduction in water movement from higher biomass would usually mean higher fine sediment composition (Van Keulen and Borowitzka 2003). This was not the case in this study with coarse sediment increasing in the impacts and finer sands decreasing. The inclusion of a mesh covering of 1 mm might trap any finer sediment within it but this size was needed to keep in macroinvertebrates. Tidal ranges, winds and other factors were within normal limits (Bureau of Meteorology) compared to previous years suggesting further studies are required to understand this.

Water quality

Results gathered from water quality showed an increase in dissolved oxygen in the impacts compared to the controls from day 18 onwards and this is due to the photosynthetic nature of *Lyngbya majuscula* and the time of day for sampling (morning)(Martinetto *et al.* 2010; Agrawal 2012). pH was generally higher in the controls and both treatments showed significant difference among sampling periods. This could be due to the high intertidal flow or natural seasonal effects. To clearly identify significant effects of *Lyngbya majuscula* on pH and D.O. % a more diurnal data would be required. However, due to the large tidal range (up to 8.35 metres between low and high tide) this could not be possible in the current study.

The changes of *Lyngbya majuscula* biomass had a significant effect on concentrations of nitrates and phosphates in the impact treatment. Once biomass density had increased to high levels on day 18 (150 g DM/m²) phosphates were depleted and showed similar trends to previous studies (Ahern *et al.* 2008; Estrella 2013). Results seemed extremely variable with the highest recorded phosphate levels being recorded one sampling period earlier than the lowest in the impact quadrats. This would suggest that more replicates would be beneficial but time constraints and low tide conditions restricted the amount of data that could be gathered for these results.

Conclusion

The in situ experiment conducted in Roebuck Bay, Western Australia has demonstrated some of the impacts that the toxic cyanobacterium *Lyngbya majuscula* can have on an enclosed macroinvertebrate assemblage. Through the use of enclosed fixed quadrats, a valid comparison was made in regard to abundance and species richness. In particular the effects *Lyngbya majuscula* had on polychaetes and gastropods. Further studies, such as long term studies at different seasonal periods and laboratory studies on anoxic/hypoxic conditions and photosynthetic rates of *Lyngya majuscula* would greatly benefit the understanding of the effects of a *Lyngbya majuscula* bloom in Roebuck Bay, Western Australia.

References

Agrawal, S. C. (2012). "Factors controlling induction of reproduction in algae - review : the text." Folia Microbiol **57**: 387-407.

Ahern, K. S. (2004). Effect of Nutrients on the Cyanobacteria Lyngbya majuscula: A Bioassay Technique. Env. Sc (Hons) Honours, The University of Queensland.

Ahern, K. S., C. R. Ahern, G. M. Savige and J. W. Udy (2007). "Mapping the distribution, biomass and tissue nutrient levels of a marine benthic cyanobacteria bloom (Lyngbya majuscula)." Marine and Freshwater Research **58**: 883-904.

Ahern, K. S., C. R. Ahern and J. W. Udy (2008). "In situ field experiment shows Lyngbya majuscula (cynaobacterium) growth stimulated by added iron, phosphorus and nitrogen." Harmful Algae **7**: 389-404.

Albert, A., J. M. O' Neil, J. W. Udy, K. S. Ahern, C. M. O' Sullivan and W. C. Dennison (2005). "Blooms of the cyanobacterium Lyngbya majuscula in coastal Queensland, Australia: disparate sites, common factors." Marine Pollution Bulletin **51**: 428-437.

Albertin, A. R. (2009). Nutrient dynamics in Florida Springs and relationship to algal blooms, University of Florida.

Alve, E. and S. T. Goldstein (2010). "Dispersal, survival and delayed growth of benthic foraminiferal propagules." Journal of Sea Research **63**: 36-51.

Arquitt, S. and R. Johnstone (2004). "A scoping and consensus building model of a toxic blue-green algae bloom." System Dynamics Review **20**(2): 179.

Arthur, K., C. J. Limpus, G. Balazs, A. Capper, J. W. Udy, G. Shaw, U. Keuper-Bennett and P. Bennett (2008). "The exposure of green turtles (*Chelonia mydas*) to tumour promoting compounds produced by the cyanobacterium *Lyngbya majuscula* and their potential role in the aetiology of fibropapillomatosis." Harmful Algae **7**: 114-125.

Arthur, K., G. Shaw, C. J. Limpus and J. W. Udy (2006). "A review of the potential role of tumour-promoting compounds produced by Lyngbya majuscula in marine turtle fibropapillomatosis." African Journal of Marine Science **28**(2): 441-446.

Arthur, K. E., C. J. Limpus, C. M. Roelfsema, J. W. Udy and G. R. Shaw (2006). "A bloom of *Lyngbya majuscual* in Shoalwater, Queensland, Australia: An important feeding ground the green turtle (*Chelonia mydas*)." Harmful Algae **5**: 251-265.

Barnes, R. D. (1982). "Invertebrate Zoology. Philadelphia, PA: Holt-Saunders International." 863–870.

Baumberger, R. E. (2008). The impacts of Harmful Algal Blooms on a Florida Reef Fish Community. Master of Science Masters, Florida Atlantic University.

Bertasi, F., M. A. Colangelo, F. Colosio, G. Gregorio, M. Abbiati and V. U. Ceccherelli (2009). "Comparing efficacy of different taxonomic resolutions and surrogates in detecting changes in soft bottom assemblages due to coastal defence structures." Marine Pollution Bulletin **58**: 686-694.

Cambridge, M. (2008). Is the increased occurrence of Lyngbya majuscula (Cyanobacteria) at Rottnest linked to higher nutrient loading? ASPAB conference 2010. Rottnest Island, Western Australia, The University of Western Australia**:** 16.

Capper, A., L. J. Flewelling and K. Arthur (2013). "Dietary exposure to harmful algal bloom (HAB) toxins in the endangered manatee (*Trichechus manatus latirostris*) and green sea turtle (*Chelonia mydas*) in Florida, USA." Harmful Algae **28**: 1-9.

Capper, A. and V. J. Paul (2008). "Grazer interactions with four species of Lyngbya in southeast Florida." Harmful Algae **7**: 717-728.

Capper, A., I. R. Tibbetts, J. M. O' Neil and G. R. Shaw (2005). "The fate of *Lyngbya majuscula* toxins in three potential consumers." Journal of Chemical Ecology **31**: 1595-1606.

Capper, A., I. R. Tibbetts, J. M. O'Neil and G. R. Shaw (2005). "The Fate of Lyngbya majuscula Toxins in Three Potential Consumers." Journal of Chemical Ecology **31**(7): 1595-1606.

Codd, G., S. Bell, K. Kaya, C. Ward, K. Beattie and M. J. S. (1999). "Cyanobacterial toxins, exposure routes and human health." European Journal of Phycology **34**(4): 405-415.

Daunys, D., D. Schiedek and S. Olenin (2000). "Species strategy near its boundary: the *Marenzelleria* cf. *viridis* (Polychaeta, Spionidae) Case in the South-Eastern Baltic Sea." Internat. Rev. Hydrobiol. **85**(5-6): 639-651.
Day, J. H. (1967). "A monograph on the Polychaeta of southern Africa." British Museum (nat. hist .) **1**.
de Goeij, P., M. Lavaleye, G. Pearson and T. Piersma (2008). Seasonal changes in the macro-zoobenthos of Roebuck Bay: A 10 year study. Report on Monitoring Roebuck Bay Benthos 1996-2005., NIOZ- Report 2008.
de Goeij, P., M. Lavaleye, G. B. Pearson and T. Piersma (2003). Seasonal changes in the macrozoobenthos of a tropical mudflat. Report on Monitoring Roebuck Bay Benthos 1996-2001, NIOZ- Report 2003-2004.
Deeley, D. M. (2009). Roebuck Bay Working Group Contingency Management Plan Broome Lyngbya, Western Australia. www.roebuckbay.org.au/pdfs/Lyngbya.
Dennison, W. C. and E. G. Abal (1999). Moreton Bay Study: a scientific basis for the Healthy Waterways Campaign. Brisbane, SEQRWQMS.
Dennison, W. C., J. M. O' Neil, E. Duffy, P. Oliver and G. Shaw (1999). Blooms of the cyanobacterium Lyngbya majuscula in coastal waters of Queensland. Proceeding of the International Symposium on Marine Cyanobacteria, Paris, Institut Oceanographique.
Escapa, M., O. Iribarne and D. Navarro (2004). "Effects of the intertidal burrowing crab *Chasmagnathus granulatus* on infaunal zonation patterns, tidal behavior, and risk of mortality." Estuaries **27**(1): 120-131.
Estrella, S. M. (2013). Effects of nutrient enrichment and toxic Lyngbya blooms on benthic invertebrates and migratory shorebird communities of Roebuck Bay Ramsar site: Final Report. The University of Western Australia.
Estrella, S. M. (2013). Roebuck Bay Lyngbya monitoring program, University of Western Australia.
Estrella, S. M., A. W. Storey, G. Pearson and T. Piersma (2011). "Potential effects of Lyngbya majuscula blooms on benthic invertebrate diversity and shorebird foraging ecology at Roebuck Bay, Western Australia: preliminary results." Journal of the Royal Society of Western Australia **94**: 171-179.
Fauchald, K. and P. A. Jumars (1979). "The diet of worms: A study of polychaete feeding guilds." Oceanography and Marine Biology: An Annual Review **17**: 193-284.
Ferris, H. and T. Bongers (2006). "Nematode Indicators of Organic Enrichment." The Journal of Nematology **38**: 3-12.
Garcia-Novoa, R. (2003). The interactions of *Lyngbya majuscula* bloom, the anthropogenic inputs and the associated meiofauna in Moreton Bay, Queensland. Masters, University of Queensland.
Garcia, R. and R. W. Johnstone (2006). "Effects of Lyngbya majuscula (Cyanophycea) blooms on sediment nutrients and meiofaunal assemblages in seagrass beds in Moreton Bay, Australia." Marine and Freshwater Research **57**(2): 155-165.
Geange, S. W. and A. C. Stier (2010). "Charismatic microfauna alter cyanobacterial production through a trophic cascade." Coral Reefs **29**: 393-397.
Geange, S. W. and A. C. Stier (2010). "Charismatic microfauna alter cyanobacterial production through a trophic cascade." Coral Reefs **29**(2): 393-397.
Giere, O. (1993). Meiobenthology: The Microscopic Fauna in Aquatic Sediments. Berlin, Springer Verlag.
Gilby, B. L., D. D. Burfeind and I. R. Tibbetts (2011). "*Lyngbya majuscula* blooms and the diet of small subtropical benthivorous fishes." Marine Biology **158**: 245-255.
Glasby, C. J., P. A. Hutchings, K. Fauchald, H. Paxton, G. W. Rouse, C. W. Russell and R. S. Wilson (2000). Polychaetes and allies: the southern synthesis. . Melbourne, Australia, CSIRO.
Grauer, F. H. and H. L. Arnold, Jr. (1961). "Seaweed dermatitis." Arch. Derm. **84**: 720-730.
Gunaratne, G. (2014). The effects of altered hydrological regimes on water quality and nutrient delivery to a sub-tropical coastal transitional wetland. PhD, University of Western Australia.
Hach (1999). HACH: DR/2010 - Spectrophotometer: Instruction Manual. USA, Hach Company.
Hale, J. and R. Butcher (2007). Ecological Character Description of the Peel-Yalgorup Ramsar Site, Report to the Department of Environment and Conservation and the Peel-Harvey Catchment Council, Perth, Western Australia.

Hamilton, G. S., F. Fielding, A. W. Chiffings, B. T. Hart, R. W. Johnstone and K. Mengersen (2007). "Investigating the Use of a Bayesian Network to Model the Risk of Lyngbya majuscula Bloom Initiation in Deception Bay, Queensland, Australia." Human and Ecological Risk Assessment **13**(6): 1271-1287.
Hamilton, G. S., R. McVinish and K. Mengersen (2009). "Bayesian model averaging for harmful algal bloom prediction." Ecological Applications **19**(7): 1805-1814.
Harr, K. E., N. J. Szabo, M. Cichra and E. J. Phlips (2008). "Debromoaplysiatoxin in Lyngbya-dominated mats on manatees (Trichechul manatus latirostris) in the Florida King's Bay ecosystem." Toxicon **52**: 385-388.
Hashimoto, Y., H. Kamiya, K. Yamazato and K. Nozawa (1976). "Occurrence of a toxic blue-green alga inducing skin dermatitis in Okinawa." Animal, Plant, and Microbial Toxins **1**: 333-338.
Huisman, J. M., F. Leliaert, H. Verbruggen and R. A. Townsend (2009). "Marine benthic plants of Western Australia's shelf-edge atolls." Records of the Western Australian Museum Supplement **77**: 50-87.
Inman, D. L. (1952). "Measures for describing the size distribution of sediments." Journal of Sedimentary Petrology **22**: 125–145.
Ismael, A. A. (2012). "Benthic bloom of cyanobacteria associated with fish mortality in Alexandria waters." Egyptian Journal of Aquatic Research **38**: 241-247.
Johnson, S., F. Fielding, G. S. Hamilton and K. Mengersen (2010). "An intergrated bayesian network approach to *Lyngbya majuscula* bloom initiation " Marine Environmental Research **69**: 27-37.
Johnstone, R. W., P. Hannington and O. Pantos (2007). *Lyngbya* Growth model, sediment and water column dynamics: The role of benthic sediments in *Lyngbya majuscula* blooms in Deception Bay. Brisbane, Australia.
Jones, A. C., L. Gerwick, D. Gonzalez, P. C. Dorrestein and W. H. Gerwick (2009). "Transcriptional analysis of the jamaicamide gene cluster from the marine cyanobacterium Lyngbya majuscula and identification of possible regulatory proteins." Bio Med Central **9**: 247-263.
Jones, A. C., E. A. Monroe, S. Podell, W. R. Hess, S. Klages, E. Esquenazi, S. Niessen, H. Hoover, M. Rothmann, R. S. Lasken, J. R. I. Yates, K. Reinhardt, M. Burkart, E. E. Allen, P. C. Dorrestein, W. H. Gerwick and L. Gerwick (2011) "Genomic insights into the physiology and ecology of the marine filamentous cyanobacterium Lyngbya majuscula." DOI: 10.1073/pnas.1101137108.
Jones, K. (2007). "Aerobic nitrogen fixation by Lyngbya sp., a marine tropical cyanobacterium." British Phycological Journal **25**(3): 287-289.
King, S. C. (2012). Effects of flow on filamentous algae and nutrient limitation in lotic systems. Doctrate of philosophy, University of Florida.
Kuffner, I. B. and V. J. Paul (2004). "Effects of the benthic cyanobacterium Lyngbya majuscula on larval recruitment of the reef corals Acropora surculosa and Pocillopora damicornis." Coral Reefs **23**: 455-458.
Langenbuch, M. and H. O. PÖrtner (2004). "High sensitivity to chronically elevated CO2 in a eurybathic marine sipunculid." Aquatic Toxicology **70**: 55-61.
M.B.R.C (2011). Moreton Bay Regional Council, Environmental Planning and Compliance Department. October 2011. Lyngbya Management Strategy. .
Mandal, S., J. Rath and S. P. Adhikary (2011). "Adaptation strategies of the sheather cyanobacterium *Lyngbya majuscula* to ultraviolet-B." Journal of Photochemistry and Photobiology **102**: 115-122.
Martın-Garcıa, L., R. Herrera, L. Moro-Abad, C. Sangil and J. Barquin-Diez (2014). "Predicting the potential habitat of the harmful cyanobacteria *Lyngbya majuscula* in the Canary Islands (Spain)." Harmful Algae **34**: 76-86.
Martinetto, P., P. Daleo, M. Escapa, J. Alberti, J. P. Isacch, E. Fanjul, F. Botto, M. Luz Piriz, G. Ponce, G. Casas and O. Iribarne (2010). "High abundance and diversity of consumers associated with eutrophic areas in a semi-desert macrotidal coastal ecosystem in Patagonia, Argentina." Estuarine Coastal and Shelf Science **88**: 357-357.
McLachlan, A. and A. Brown (2006). The Ecology of Sandy Shores. Amsterdam, Elsevier.
Metcalfe, K. and C. J. Glasby (2008). "Diversity of Polychaeta (Annelida) and other worm taxa in mangrove habitats of Darwin Harbour, Northern Australia." Journal of Sea Research **59**: 70-82.
Metcalfe, K. and C. J. Glasby (2008). "Diversity of Polychaete (Annelida) and other worm taxa in mangrove habitats of Darwin Harbour, Northern Australia. ." Journal of Sea Research **59**: 70-82.

Nagle, D. G., V. J. Paul and M. Roberts (1996). "Ypaoamide, a New Broadly Acting Feeding Deterrent from the Marine

Cyanobacterium Lyngbya majuscula." Tetrahedron Letters **37**(35): 6263-6266.
Ng, C. S. L., L. J. Ong and L. M. Chou (2012). "*Lyngbya majuscula* blooms in an enclosed marine environment." Environment Asia **5**(2): 93-98.
Nobes, K., S. Uthicke and R. Henderson (2008). "Is light the limiting factor for the distribution of benthic symbiont bearing foraminifera on the Great Barrier Reef?" Journal of Experimental Marine Biology and Ecology **363**: 48-57.
O' Neil, J. M., T. W. Davis, M. A. Burford and C. J. Gobler (2012). "The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change." Harmful Algae **14**: 313-334.
O'Brien, A. L., L. Morris and M. J. Keough (2010). "Multiple sources of nutrients add to the complexities of predicting marine benthic community responses to enrichment." Marine and Freshwater Research **61**: 1388-1398.
Osborne, N. J., A. Seawright and G. Shaw (2008). "Dermal toxicology of *Lyngbya majuscula*, from Moreton Bay, Queensland, Australia." Harmful Algae **7**: 584-589.
Osborne, N. J., G. R. Shaw and P. M. Webb (2007). "Health effects of recreational exposure to Moreton Bay, Australia waters during a *Lyngbya majuscula* bloom." Environment International **33**: 309-314.
Osborne, N. J., P. M. Webb and G. R. Shaw (2001). "The toxins of Lyngbya majuscula and their human and ecological health effects." Environment International **27**: 1–12.
Paerl, H. W., K. M. Crocker and L. E. Prufert (1987). "Limitation of nitrogen fixation in coastal marine waters: relative importance of molybdenum, iron, phosphorus and organic matter availability." Limnology and Oceanography **32**: 525–536.
Paerl, H. W. and T. G. Otten (2013). "Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls." Microb Ecol **65**: 995-1010.
Paerl, H. W. and V. J. Paul (2012). "Climate change: Links to global expansion of harmful cyanobacteria." Water Research **46**: 1349-1363.
Paul, V. J., K. E. Arthur, R. Ritson-Williams, C. Ross and K. Sharp (2007). "Chemical defences: from compounds to communities." Biological Bulletin **213**: 226-251.
Paul, V. J., R. W. Thacker, K. Banks and S. Golubic (2005). "Benthic cyanobacterial bloom impacts the reefs of South Florida (Broward County, USA)." Coral Reefs **24**: 693-697.
Pessoa, M. F. (2012). "Algae and aquatic macrophytes responses to cope to ultraviolet radiation- a Review." Emir. J. Food. Agric. **24**(6): 527-545.
Piersma, T., G. B. Pearson and M. Lavaleye (1998). "The teeming mud of Roebuck Bay." Landscope **13**: 16-22.
Pittman, S. J. and K. M. Pittman (2005). "Short-term consequences of a benthic cyanobacterial bloom (Lyngbya majuscula Gomont) for fish and penaeid prawns in Moreton Bay (Queensland, Australia)." Estuarine, Coastal and Shelf Science **63**: 619-632.
Postgate, J. R. (1987). Nitrogen Fixation, Edward Arnold.
Potts, M. (1994). "Desiccation tolerance of prokaryotes." Microbiological Research **19**: 319-342.
Raffaelli, D. G., J. A. Raven and L. J. Poole (1998). "Ecological impact of green macroalgal blooms." Oceanography and Marine Biology: An Annual Review **36**: 97-125.
Rogers, D., T. Piersma, M. Lavaleye, G. Pearson and P. de Goeij (2003). Life along land's edge. Wildlife on the shores of Roebuck Bay, Broome., Department of Conservation and Land Management, Western Australia.
Steyeart, M., P. M. J. Herman, T. Moens and M. Vincx (2003). "Variation and depth distribution of a nematode community during a tidal cycle (Molenplaat, Westerschelde estuary, The Netherlands)." Marine Ecology Progress Series **224**: 229-304.
Stielow, S. and D. L. Ballantine (2003). "Benthic cyanobacterial, Microcoleus lyngbyaceus, blooms in shallow, inshore Puerto Rican seagrass habitats, Caribbean Sea." Harmful Algae **2**: 127-133.
Taylor, M. S., W. Stahl-Timmins, C. H. Redshaw and N. J. Osborne (2014). "Toxic alkaloids in *Lyngbya majuscula* and related tropical marine cyanobacteria." Harmful Algae **31**: 1-8.

Uthicke, S. and K. Nobes (2008). "Benthic Foraminifera as ecological indicators for water quality on the Great Barrier Reef." Estuarine, Coastal and Shelf Science **78**: 763-773.
Van Keulen, M. and M. A. Borowitzka (2003). "Seasonal variability in sediment distribution along an exposure gradient in a seagrass meadow in Shoalwater Bay, Western Australia." Estuarine, Coastal and Shelf Science **57**: 587-592.
Vaquer-Sunyer, R. and C. M. Duarte (2008). "Thresholds of hypoxia for marine biodiversity." Proceedings of the National Academy of Sciences **40**: 5452-15457.
Vogwill, R. I. J. (2003). Hydrogeology and Aspects of the Environmental Geology of the Broome Area Western Australia, PhD Thesis, Curtin University, Western Australia.
Watkinson, A. J., J. M. O'Neil and W. D. Dennison (2005). "Ecophysiology of the marine cyanobacterium, Lyngbya majuscula (Oscillatoriacea)." Journal of Harmful Algae **4**: 697-715.
Wear, R. G. and J. P. A. Gardner (2001). "Biological effects of the toxic algal bloom of February and March 1988 on the benthos of Wellington Harbour, New Zealand." Marine Ecology Progress Series **218**: 63-76.
Yates, D. S., D. S. Moore and D. S. Starnes (2008). The Practice of Statistics, 3rd Ed. , Freeman.