The ecology and feeding biology of the sponge *Rhopaloeides odorabile*

Thesis submitted by

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July 2008

for the degree of Doctor of Philosophy
in the School of Marine and Tropical Biology
James Cook University

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Research funding for this PhD study was provided through the Australian Institute of Marine Science, James Cook University Research Advancement Program – Finfish and Emerging Aquaculture, CRC Reef. A James Cook University Postgraduate Award provided stipend support.

Acknowledgements

First and foremost, I would like to acknowledge both of my supervisors, Professor Rocky de Nys and Dr Chris Battershill. Throughout the duration of my candidature, Rocky has been an excellent supervisor, providing enthusiasm, endless support and an open attitude, which has resulted in a remarkable student-mentor relationship. Thank you for your encouragement and high motivation, without which the completion of this PhD thesis would not have been possible. Chris, thank you for many delightful discussions on sponge ecology and providing me with a greater understanding of experimental design and statistical analysis. Your support and assistance with underwater field surveys and sample collection whilst at sea was also invaluable. Also, thank you to Dr Raphael Wust. He always had his door open for me when I needed advice. He also provided interesting discussions on marine sediments and their impacts on benthic habitats, sparking future research ideas and topics.

I would also like to thank all members of the de Nys lab over the past four years, especially Steve Whalan, Nick Paul, Piers Ettinger-Epstein, Andrew Scardino and Michael Horne, who have provided in-depth scientific discussions and an enjoyable working environment. Thank you to the many volunteers that have helped me in the field and the laboratory. I would especially like to thank all my diving volunteers that have spent countless hours underwater with me and many more putting up with my fading sense of humour during extended periods of time isolated at Orpheus Island Research Station. These volunteers included Rod Hidlebaugh, May-Helen Holme, Adam Mills, Steve Whalan, David Bourne, Rebecca Fox, Dommie Loong, Andy Cole, Daniel Louden, Chris Ryen, Kate Markey, Carsten Wolff and Alan Duckworth.

Thank you to several researchers at James Cook University and other institutions, who have provided me with technical assistance or access to scientific equipment to collect and analyse my data. These people included Dr Raphael Wust, Dr Heiner Korner, Dr Kirsten Heimann, Dr Joe Holtum, Dr Adele Pile, Dr Ken Anthony, Dr Mia Hoogenboom, Professor Mike Kingsford and the Advanced Analytical Center (AAC) at James Cook University. Thank you to all the staff at Orpheus Island Research Stations, especially, Anna, Ollie and Pete who would literally bend over backwards to fulfil my field trip requirements. I would also like to thank all the crew onboard the research vessels Cape Ferguson, and the recently decommissioned Lady Basten, who provided assistance throughout the duration of each cruise.

I would also like to thank Dr Nick Paul and Dr Jana Guenther for their assistance with statistical analysis, which was very much appreciated.

A special thank you to Rod Hidlebaugh, Rocky de Nys, Nick Paul, Andrew Scardino and Chris Glasson for the weekly competitive golf games. These games provided some comic relief from the stresses of being a PhD student.

Finally, I would like to thank my family. My beautiful fiancé Jana Guenther has supported me whole heartily throughout my candidature, despite my frequent 12-day trips away in the field. She has provided me with much love and support and an enormous amount of encouragement over the last four years, and without her by my side this thesis would have been an even greater uphill battle. I would also like to thank my parents, Steve and Rhonda, for their support and encouragement throughout my studies.

Abstract

On large coral reef systems like the Great Barrier Reef, with a significant cross shelf component throughout most of its length, sponges inhabit a broad range of benthic habitats, from shallow water turbid coastal reefs to clear water oceanic reef systems. The structure of sponge assemblages differs taxonomically and morphologically across these varied benthic habitats, responding to environmental conditions and reflecting the prevailing biophysical character. Despite these differences sponges as a group constitute a critical component of the benthos across all these habitats, providing structural rigour, and contributing to benthic-pelagic nutrient fluxes. Sponges also provide a substrate for the settlement and refuge of other benthic organisms and are a host to a large diversity and biomass of symbiotic microorganisms. Despite the importance of sponges within these habitats our understanding of population distributions and the key environmental parameters that influence and maintain them is in its infancy. This knowledge is fundamental to conservation and management of marine benthic environments.

The abundance, size and depth distribution of the sponge *Rhopaloeides odorabile* was quantified within inner-, mid- and outer-shelf reef locations across the continental shelf of the central Great Barrier Reef (GBR), Australia using belt transects. There was a clear gradient in the abundance, size and depth distribution of *R. odorabile* across all reefs sampled, with mean abundances on mid- (20.3 ± 2.2 individuals.250 m^{-2}) and outer-shelf reefs (22.6 ± 6.4 individuals.250 m^{-2}) being more than three times greater than inner-shelf reefs (6.2 ± 2.4 individuals.250 m^{-2}). In addition, although not statistically different, the mean size of *R. odorabile* on outer-shelf reef locations (4236.3 ± 941.9 cm^3) were larger than *R. odorabile* on inner- (3096.3 ± 1394.2 cm^3) and mid-shelf reef locations (2323.8 ± 548.3 cm^3), with *R. odorabile* preferring to inhabit deeper habitats on mid- and

outer-shelf reef locations (8 to 12 m) than inner-shelf reef locations (<8 m). These distinct differences between shelf locations may be driven by differences in environmental gradients across shelf locations, in particular light availability, sedimentation and food availability.

Variation in light, sediment and food are key factors structuring the distribution, abundance and physiology of many sessile marine invertebrates in benthic habitats, particularly those harbouring symbiotic (possibly phototrophic) microbial symbionts. Photophysiology results for *R. odorabile* individuals from inner-, mid-, and outer-shelf reef locations, demonstrate that light availability does not physiologically regulate the size and depth distributions of *R. odorabile* across shelf locations. Photophysiology experiments identified that regardless of their origin, *R. odorabile* individuals collected across inner-, mid-, and outer-shelf reef locations did not photosynthesise or possess any photopigments. Therefore, *R. odorabile* does not acquire energy requirements by way of photosynthesis, showing that in terms of energy acquisition light availability does not influence size and/or the depth distribution of *R. odorabile* across shelf locations.

To quantitatively determine the impact of sedimentation and the role of sediment grain size and mineralogy on the ecology and physiology of *R. odorabile*, suspended sediment was sampled monthly across winter (Austral tropical dry season) and summer (Austral tropical wet season) at different locations including Pelorus Island (inner-shelf reef), Rib Reef (mid-shelf reef), and Pith Reef (outer-shelf reef). Regardless of month sampled, up to 32% of the volume of sediment on inner-shelf reefs was dominated by fine clay sediments (mean grain size 24.4 ± 5.7 μm), in contrast to mid and outer shelf reefs where less than 3% of the volume of sediments were fine clay sediments and instead sediments are dominated by coarse biogenic materials (mean grain sizes of 126.6 ± 30.5 μm and 214.3 ± 33.4 μm, respectively) (carbonate sediments). Decreasing sediment grain size and increasing clay content of suspended sediments towards the coast corresponded with the decreasing abundance, size and reduced depth distribution of *R. odorabile* towards the coast.

Food availability and the retention efficiency of ultraplankton cell types <3 μm were quantified for *R. odorabile* across inner-, mid- and outer-shelf locations. Across these shelf locations, *R. odorabile* was exposed to a broad range of ultraplankton cell types, including heterotrophic bacteria, *Prochlorococcus* spp., *Synechococcus* – type cyanobacteria and picoeukaryotes <3 μm. Food availability was more abundant on inner-shelf locations than outer-shelf locations, including picoeukaryotes <3 μm, the main planktonic food source for *R. odorabile*. Picoeukaryotes were retained preferentially with high efficiencies and these efficiencies are consistent across shelf locations (inner-shelf, 95%; mid-shelf, 87%; outer-shelf, 92%). Given the higher abundance of picoeukaryotes <3 μm on inner-shelf reefs, *R. odorabile* at these reefs assimilated 1.5 times more carbon (8.38 ± 0.10 μg $C.l^{-1}$) and nitrogen (1.34 ± 0.02 μg $N.l^{-1}$) than on mid-shelf reefs (5.23 ± 0.84 μg $C.l^{-1}$, 0.84 ± 0.13 μg $N.l^{-1}$), and 3 times more carbon and nitrogen than outer-shelf reefs (2.71 ± 0.90 μg $C.l^{-1}$, 0.44 ± 0.15 μg $N.l^{-1}$). Paradoxically, *R. odorabile* retains significantly more carbon and nitrogen in the form of picoeukaryotes on inner-shelf reefs than mid- and outer-shelf reefs, however, *R. odorabile* are not larger or more abundant on inner-shelf reefs. This may be explained by a metabolic 'trade-off' from increased energetic costs for *R.odorabile* living in high sediment inshore environments exposed to fine clay sediments.

Experimental manipulation of sediment grain size and mineralogy on the respiration rate of *R. odorabile* demonstrated unequivocally that fine clay sediments (mean grain size 3.1 ± 0.1 μm) increase the respiration activity from baseline respiration rates by 35% during short-term exposure (7 hours) and up to 43% during long-term exposure (4 days). Visual observations also identified that exposure to fine clay sediments induce the production of mucus on the external surfaces of *R. odorabile*, whilst the shutdown of sponge oscula was also evident. Therefore, exposure of *R. odorabile* on inner-shelf reefs to fine clay suspended sediments that are absent on mid- and

outer-shelf reefs increases their metabolic costs, which may be responsible for reduced abundance and sizes of *R. odorabile* on inner-shelf reefs. This suggests that inner-shelf reefs provide sub-optimal habitat conditions for *R. odorabile*. These higher energetic drains in inshore environments may result in energy normally utilised for growth and reproduction being diverted to maintenance and survival, resulting in lower reproductive output, smaller individuals and subsequently lower abundance. These findings highlight the importance of identifying the key environmental parameters linked with anthropogenic change on the dynamics and structure of an important component of the benthic community.

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Chapter 1 – General Introduction

1.1 Sponges – structure and diversity

Sponges (Phylum Porifera) are a diverse group of sessile filter feeding metazoans classified into four classes: Calcarea, Hexactinellida, Demospongiae and Sclerospongiae (Bergquist 1978; Hooper and van Soest 2002). Currently, more than 8,000 valid species of sponges have been taxonomically identified with 95% of these species being classified within the class Demospongiae (Hooper and van Soest 2002; van Soest et al. 2005). Estimates on sponge biodiversity exceed 15,000 species globally (Hooper and Lèvi 1994; Hooper et al. 2002). Sponges are multicellular animals, functioning without organs and true tissues, instead relying on a system of water canals (known as the aquiferous system) and a suite of specialised mobile cells to carry out bodily functions, including feeding, respiration and reproduction (Bergquist 1978; Simpson 1984). Despite this

simplistic body plan marine sponges can possess a diverse range of morphologies, these include, but are not limited to encrusting, foliose, massive and branching forms (Barnes and Bell 2002). Furthermore, individual sponge species are capable of changing their gross morphology to adapt to their environment, presumably to optimise respiration, feeding and the dispersal of reproductive and waste products (Palumbi 1984; Kaandorp 1999; McDonald et al. 2002, 2003).

In addition to morphological plasticity many sponges have also developed close associations with microbial symbionts (reviewed in Taylor et al. 2007a). These symbiotic associations are presumably beneficial to the host, providing many sponges with their nutritional requirements, either photosynthetically or through the fixation of carbon and nitrogen by other forms of nutritional symbiosis (reviewed in Taylor et al. 2007a). Sponge-microbial symbioses also provide several other beneficial interactions for sponges, including enhanced growth rates, UV protection, removal of toxic metabolic by-products, and defence against fouling organisms, predators and pathogens through the production of biologically active secondary metabolites (reviewed in Taylor et al. 2007a).

Cell mobility, tactile morphological acclimation and the association of diverse microbial symbionts have allowed sponges to exploit many different habitat types. More than 8,000 species of sponges are distributed from shallow water temperate and tropical reefs habitats, to deep-sea polar benthic habitats (Hooper and van Soest 2002; van Soest et al. 2005). Within these different habitat types, sponges are important components of the benthic biota, transferring energy from pelagic to benthic habitats (Reiswig 1971a; Pile et al. 1996; Pile and Young 2006), providing structural rigour to reef frameworks (Wulff and Buss 1979; Wulff 1984, 2001), substrate for settlement (Barthel and Gutt 1992; Bett and Rice 1992) and refuge for other organisms (reviewed in Wulff 2006; Abdo 2007).

Despite the dominance of sponges in many habitat types, the structure and composition of sponge communities can vary spatially (Wilkinson and Cheshire 1989; Hooper and Kennedy 2002; Hooper et al. 2002). Species specific responses of sponges to a suite of biological and environmental factors may be responsible for changes to sponge assemblages across different spatial scales (Hooper and Kennedy 2002). These factors include sediment (Zea 1994; Bell and Smith 2004; Cleary et al. 2005; Carballo 2006), light (Wilkinson and Trott 1985; Jokiel 1980), food availability (Storr 1976; Wilkinson and Cheshire 1989; Cleary et al. 2005; Lesser 2006), larval dispersal and recruitment (Zea 1993; Maldonado and Young 1996, 1998), exposure (Wilkinson and Evans 1989; Bannister et al. 2007), substrate type and angle (Bell and Barnes 2000a,b), pollution (Cebrian and Uriz 2007a,b), competition and predation (reviewed in Wulff 2006).

The morphological and physiological responses of sponges to environmental variables have a strong influence on community structure. For example, light availability and sedimentation are suggested to be key determinates of phototrophic sponge population structure, influencing the distribution of species between coastal and offshore habitats (Wilkinson and Cheshire 1989; Cleary et al. 2005). Similarly, increased food availability is documented to drive increased abundance and distribution of heterotrophic sponges in coastal habitats compared with offshore habitats (Wilkinson and Cheshire 1989; Cleary et al. 2005). Broad scale distribution and abundance patterns of sponge assemblages can be recorded. However, an increased understanding of the factors driving observed patterns in biogeography and population structure within regions is necessary to permit quantification and prediction of the impacts of anthropogenic change on the marine environment, and to identify those that have the greatest impact.

1.2 Environmental factors affecting distribution and abundance of sponges

1.2.1 Sediment

Sedimentation is a key environmental parameter influencing sponge assemblages across many different habitat types. Increased rates of sedimentation influence the structure, composition (Zea 1994; Bell and Barnes 2000a,b; Carballo 2006) and morphology of sponge assemblages (Maldonado and Uriz 1999; Cerrano et al. 2002; McDonald et al. 2002; Bell 2004), and is documented to impede growth (Lohrer et al. 2006; Roberts et al. 2006) and reproduction (Roberts et al. 2006; Whalan et al. 2007a), whilst reducing pumping activity (Gerrodette and Flechsig 1979) and the clearance rates of sponges (Lohrer et al. 2006). The majority of studies examining the negative impacts of suspended sediments on sponges measure the total weight of sediment (deposition rate). However, these studies have disregarded grain size and the mineralogy of suspended sediments. This is surprising, given a plethora of studies indicating that the physical, mineralogical and organic properties of suspended sediments are the primary factors responsible for physiological stress responses in corals (reviewed in Fabricius 2005; Weber et al. 2006). Therefore, elucidating the physical, mineralogical and organic properties of suspended sediments in sponge habitats is necessary to further delineate the effects of suspended sediments and their role in structuring sponge assemblages in benthic habitats.

While the impact of sedimentation has been identified as a major factor structuring sponge assemblages and morphologies across many different habitat types (Carballo et al. 1996; Bell and Barnes 2000a,b; Bell 2004; Carballo 2006), the effects of sediments on sponge physiology are still poorly understood. Excessive fine clay sediments clog inhalant canals and filtering structures of sponges resulting in the shut down of pumping activity (Gerrodette and Flechsig 1979), and the production of mucus to trap and remove sediments (Turon et al. 1999). Although reduced pumping activity of sponges has been suggested to impede feeding and reduce respiration rate of sponges

(Resiwig 1974; Gerrodette and Flechsig 1979), experimental evidence elucidating the impact of suspended sediments on sponge physiology is lacking. Similarly, while the production of mucus to deter the deleterious effects of sedimentation can be costly for corals, the energetic costs of mucus production by sponges are unknown. Due to insufficient experimental evidence testing a broad spectrum of sediment grain sizes and mineralogical compositions, further examination is required to elucidate the role of suspended sediment grain size and mineralogy on the feeding and respiration of sponges. These results may in turn help determine their presence and demographics, or absence in a particular habitat.

1.2.2 Light

Light is an important environmental factor affecting sponges with and without photosymbionts. For phototrophic sponges, the availability of light is crucial for photosynthetic processes contributing to energy acquisition and growth (Wilkinson and Vacelet 1979; Wilkinson 1983; Thacker 2005). In addition, light availability may also assist in larval dispersal and settlement, regulating the distribution and abundance patterns of marine sponges (reviewed in Maldonado 2006; Whalan et al. in press). The distribution of sponges in response to light availability may enhance energy acquisition (Wilkinson 1979, 1983), sponge growth (Wilkinson and Vacelet 1979; Hill 1996) and boring rates (Hill 1996), whilst providing protection against UV damage (Jokiel 1980) and oxidative stress in sponges (Regoli et al. 2000). Furthermore, light may also aid in secondary metabolite production by photosynthetic symbionts to deter predation and alleviate competitive interactions with other spatial competitors (Becerro and Paul 2004). Therefore, light availability plays an important role in the ecology of sponges, from larval settlement to the protection of established individuals against predation and competition. This highlights the importance of understanding the role of light in determining distribution and abundance patterns of sponges.

1.2.3 Food availability

In terms of growth and reproduction, food availability is a critical environmental factor influencing the dynamics of benthic marine invertebrates (Reviewed in Coma and Ribes 2003). For marine sponges, food availability has been suggested to be a key factor influencing the structure and dynamics of sponge communities (Storr 1976; Wilkinson and Cheshire 1989; Tanaka 2002; Lesser 2006). Recently, it has been demonstrated that food availability is a key factor affecting the growth and subsequently the abundance of sponges (Lesser 2006; Trussell et al. 2006), however, few studies have investigated food availability as a constraining factor for sponges in coral reef habitats. This is despite, the distinct distribution and biomass patterns of sponges across cross shelf coral reef systems (Wilkinson and Cheshire 1989; Cleary et al. 2005) and their exposure to distinct differences in cross-shelf phytoplankton composition, biomass and community dynamics across these reef systems (Revelante et al. 1982; Furnas and Mitchell 1986).

Sponges are highly efficient filter feeders (Reiswig 1971a; Pile et al. 1996) and employ diverse strategies to meet their nutritional requirements. Sponges filter and retain phytoplankton (Ribes et al. 1999a), ultraplankton (Reiswig 1971a; Pile et al. 1996; Pile 1997; Pile and Young 2006) and viruses with high efficiencies (Hadas et al. 2006). Furthermore, in association with their microbial symbionts, sponges also assimilate dissolved organic carbon (DOC) (Reiswig 1990; Yahel et al. 2003; de Goeij et al. 2008; van Duyl et al. 2008) and photosynthetically derived carbon from symbiotic cyanobacteria (Wilkinson 1979; Wilkinson 1983). Additionally, microbial symbionts can also contribute to other nutritional requirements of sponges, including nitrogen fixation and the consumption of methanotrophs (reviewed in Taylor et al. 2007a). This plasticity in sponge feeding may explain why sponges can exploit many different habitat types (Ribes et al. 1999a). Sponges can also change their feeding efficiencies both temporally (Ribes et al. 1999a) and spatially (Bell et al. 1999). However, there is a lack of experimental evidence detailing spatial variation in sponge

feeding or the mechanisms that drive these patterns. This is surprising given the ability of sponges to occupy different microhabitats across varying spatial scales exposed to different environmental factors (Wilkinson and Cheshire 1989; Wilkinson and Evans 1989).

1.3 Thesis aims

This thesis aims to address the relative importance of key environmental factors, including light, sediment and food availability associated with a water quality gradient in structuring the distribution and abundance of sponges across inner-, mid- and outer-shelf locations of the central Great Barrier Reef (GBR). A model species was selected with a strong background of biological information and with a known large-scale biogeographic distribution. This allows the examination of a clearly defined ecological continuum affected by a distinct cross shelf environmental gradient. The study sponge *Rhopaloeides odorabile* Dictyoceratida, Spongiidae (Thompson, Murphy, Bergquist and Evans 1987) is a common sponge with a broad distribution across shelf locations of the central GBR (Wilkinson and Cheshire 1989). *R. odorabile* has a massive, amorphous morphology, with distinct oscula positioned on the dorsal ridge and is also characterised by a red-brown surface pigmentation in illuminated habitats (Thompson et al. 1987) (Figure 1.1). Assessment of both the reproductive (Whalan et al. 2007a,b, Whalan et al. in press) and microbial ecology (Webster and Hill 2001; Webster et al. 2001a,b; Webster et al. 2002) have recently been undertaken for this species.

Figure 1.1 The sponge *Rhopaloeides odorabile*

A comprehensive survey of the distribution and abundance of *R. odorabile* was carried out across the continental shelf of the central GBR. In addition, *in situ* sampling was carried out to elucidate the size and mineralogy of suspended sediment properties and the feeding ecology of *R. odorabile*, across inner-, mid- and outer-shelf locations. Furthermore, manipulative experiments were undertaken to quantify the role of light and sediment influencing metabolic processes of *R. odorabile* from inshore to offshore shelf environments. The combination of these data will identify the key environmental factors structuring benthic sponge communities. This thesis is presented in five chapters that address the distribution and abundance (Chapter 2), photophysiology (Chapter 3), feeding ecology (Chapter 5) and the impact of suspended sediments (Chapter 4 & 6) on populations of *R. odorabile* on the central GBR.

In Chapter 2, extensive field surveys were conducted to quantify the distribution, abundance and size patterns of *R. odorabile* across an environmental gradient (i.e. water quality) from inshore (within 20 km from the coast) to offshore reef habitats (>60 km from the coastline) on the central

GBR. Results provide key information on the ecology of *R. odorabile*, highlighting distinct differences in abundance, size and depth distributions between coastal and offshore reef habitats. This provides a critical first step towards identifying the parameters that influence the distribution and abundance of *R. odorabile*.

Chapter 3 quantifies the photophysiological potential of *R. odorabile* individuals from inner-, mid- and outer-shelf locations spanning the environmental gradient across the GBR shelf. Oxygen evolution experiments and pigment analysis were used to identify and compare photosynthesis in *R. odorabile* collected across shelf locations.

Chapter 4 investigates the composition and structure of suspended sediments, in particular sediment grain size and mineralogy, across inner-, mid- and outer-shelf habitats of *R. odorabile.* Comparisons are drawn between suspended sediment grain size and mineralogy across inner-, mid- and outer-shelf locations and the distinct distribution and abundance patterns of *R. odorabile* across shelf locations.

Chapter 5 quantifies spatial and temporal variation in the ultraplankton component of the diet of *R. odorabile*. This chapter determines the four ultraplankton cell types retained by *R. odorabile* across the environmental gradient on inner-, mid- and outer-shelf reef locations during three different months. Temporal and spatial comparisons are made between the retention efficiencies of ultraplankton and changes in ultraplankton abundance, as well as distinct variations in the exposure to suspended sediment grain sizes and mineralogy between different sponge habitats across shelf locations.

Chapter 6 expands on the data collected in Chapter 4 and quantifies the physiological responses of *R. odorabile* to suspended sediments of contrasting mineralogical composition. This chapter

measures short-term and longer-term respiratory responses of *R. odorabile* exposed to fine clay and carbonate sediments of similar size at ecologically relevant concentrations.

The results of this study are synthesised and discussed in Chapter 7, providing a broad overview of the key factors affecting the structure of *R. odorabile* populations across shelf locations of the central GBR. The chapter also addresses directions for future research.

Chapter 2 – Abundance, Size and Distribution of *Rhopaloeides odorabile*: A Cross Shelf Comparison

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2.1 Introduction

Environmental gradients on cross shelf reef systems contribute to variations in the diversity, size-frequency composition and overall abundance of organisms within sessile marine assemblages from inshore to offshore reef environments (Wilkinson and Cheshire 1989; Adjeroud 1997; Cleary et al. 2005; Cleary and de Voogd 2007). The influence of terrigenous runoff and associated sediment, nutrients and pollutants are important factors establishing these environmental gradients (Fabricius and De'ath 2001; Fabricius et al. 2005), with inner-shelf reefs (within 20 km of the coastline) experiencing water of poorer quality compared to mid- and outer-shelf reefs that are increasingly influenced by oceanic waters (Devlin and Brodie 2005).

While the effect of environmental gradients on tropical reef systems have been investigated in some detail for corals (reviewed in Fabricius 2005) little is known of the influence of environmental gradients on sponges, a phylogenetic group likely to reflect prevailing biophysical conditions in their demographics, morphology and species assemblage at any particular location. This is a considerable gap in the literature given sponges form an integral component of tropical reef systems (Reiswig 1973; Wilkinson and Cheshire 1989; Alvarez et al. 1990; Schmahl 1990; Long et al. 1995; Diaz and Rützler 2001) where they are reported to establish distinct cross shelf distribution and biomass patterns (Wilkinson and Trott 1985; Wilkinson and Cheshire 1989; de Voogd et al. 1999; Cleary et al. 2005; Cleary and de Voogd 2007). However, these patterns are only anecdotally linked with variations in physical and biological factors across the reef shelf.

On the central GBR, richness and biomass patterns of sponges are well structured with inner-shelf reefs supporting high sponge diversity and biomass (Wilkinson and Cheshire 1989). In comparison, outer-shelf reefs are taxonomically different maintaining lower species diversity with considerably lower biomass (Wilkinson and Cheshire 1989). Heterotrophic sponges dominate inshore reefs, whereas sponges offshore are predominately phototrophic (Wilkinson and Trott 1985; Wilkinson and Cheshire 1989). These distinct patterns between two geographically different reef environments are proposed to be due to the water quality gradients established across the reef shelf (Wilkinson and Cheshire 1989), with inshore reefs experiencing higher levels of sedimentation, nutrients and substantially reduced light levels compared to mid- and outer-shelf reefs (Devlin and Brodie 2005; Udy et al. 2005). Whilst factors influencing broad scale patterns of sponge assemblages are clear (Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006), a greater understanding of factors impacting on the distribution and abundance patterns of individual species is required. This is not only important to understand the key driving factors affecting community composition, but also to quantify the effects of anthropogenic change on the marine environment and the mechanisms that have the greatest impacts.

One species of sponge distributed across the reef shelf on the central GBR is *R. odorabile* (Wilkinson and Cheshire 1989), and it provides the opportunity to develop a model to investigate the effects and components of environmental gradients on the distribution and abundance of sponges. *R. odorabile* has a massive morphology and a clear preference for high-energy, oligotrophic environments (Wilkinson and Evans 1989). On mid-shelf reefs, *R. odorabile* colonises fore reef sites at depths between 5 and 15 m, where strong wave induced turbulence is a regular feature (Wilkinson and Evans 1989). A similar pattern is found on inner-shelf reefs where the distribution of *R. odorabile* is heterogeneous with increased abundance in exposed habitats with strong currents and regular wave activity (Bannister et al. 2007). Although there is a preference for high-energy environments, the abundance of *R. odorabile* is paradoxically slightly higher on inner-shelf reefs (Wilkinson and Cheshire 1989), which are generally considered low-energy environments. Understanding the factors affecting the distribution, abundance and size patterns of *R. odorabile* across environmental gradients of the GBR will improve our knowledge of the relative importance of the various biological and physical elements of environmental gradients on influencing the distribution and abundance of sponges. However, most importantly, it provides a model to quantify the impacts of critical gradients affected by human activities, such as salinity (runoff), nutrients (eutrophication), food availability (eutrophication) and sediments (runoff), on the composition of benthic communities.

In earlier research, Wilkinson and Cheshire (1989) examined the abundance and biomass patterns of *R. odorabile* across the reef shelf of the central GBR, focusing on variations in sponge community structure over broad spatial scales, identifying population parameters to characterise reef systems or habitats. Surveys were limited to individual sites of reefs within each shelf location (inner-, mid-, outer-shelf reefs) and covered limited reef substrate at each depth surveyed. Given the distribution and abundance of *R. odorabile* is patchy on small and large spatial scales on inner-shelf reefs of the

central GBR (Bannister et al. 2007), it is appropriate to survey a greater number of larger transects at multiple sites at each reef to accurately quantify distribution, abundance and size patterns of *R. odorabile* across shelf locations. This also provides an opportunity to make qualitative comparisons between the distribution and abundance of *R. odorabile* twenty years ago, given the changes in water quality and the well documented environmental gradients that have been established across the GBR coral reef system (Devlin and Brodie 2005; Udy et al. 2005).

This chapter quantifies the abundance and size of *R. odorabile* across the continental shelf of the central GBR. It specifically addresses the following: (1) Is there a difference in the abundance of *R. odorabile* across inner-, mid- and outer-shelf reefs? (2) Does the average size of *R. odorabile* change with respect to their position on the reef shelf? (3) Does the population size structure of *R. odorabile* change across inner-, mid- and outer-shelf reefs?

2.2 Materials and methods

2.2.1 Study sites

Underwater surveys were conducted during January and February 2005 on SCUBA to measure the abundance, size and distribution of *R. odorabile* across inner-, mid- and outer-shelf reef locations of the central GBR. Inner-shelf reefs are located within 20 km from the coastline, mid-shelf reefs between 40 to 60 km from the coastline and outer-shelf reefs are beyond 60 km from the coastline. These distance classifications for reef shelf locations are the same as those used by Wilkinson and Cheshire (1989).

Surveys were conducted on the inner-shelf fringing reefs of Dunk Island (17°58.19’S, 146°10.49’E), Pelorus Island (18°33.85’S, 146°29.93’E) and Curacoa Island (18°40.27’S, 146°34.06’E). The mid-shelf reefs were Davies Reef (18°49.50’S, 147°39.46’E), Kelso Reef

(18°26.75'S, 147°00.34'E) and Small Kelso Reef (18°27.99'S, 147°00.33'E). The outer-shelf reefs were Fork Reef (18°35.37'S, 147°34.03'E), Dip Reef (18°24.79'S, 147°26.92'E) and Pith Reef (18°13.26'S, 147°01.58'E) (Figure 2.1).

All surveys took place at exposed locations (south east face, proximal to the major south east trade winds and wave inputs) at each reef as *R. odorabile* prefers high-energy environments that have good water circulation and strong currents (Wilkinson and Evans 1989; Bannister et al. 2007). Environmental parameters that differ between inner-, mid- and outer-shelf reef locations on the central GBR include light availability (Anthony et al. 2004; Udy et al. 2005), currents (Church et al. 1985; Wolanski and Pickard 1985), nutrients and sedimentation (Devlin and Brodie 2005). Furthermore, sediment properties (sediment grain size and mineralogy, Chapter 4) and food availability also change across the reef shelf locations (Chapter 5).

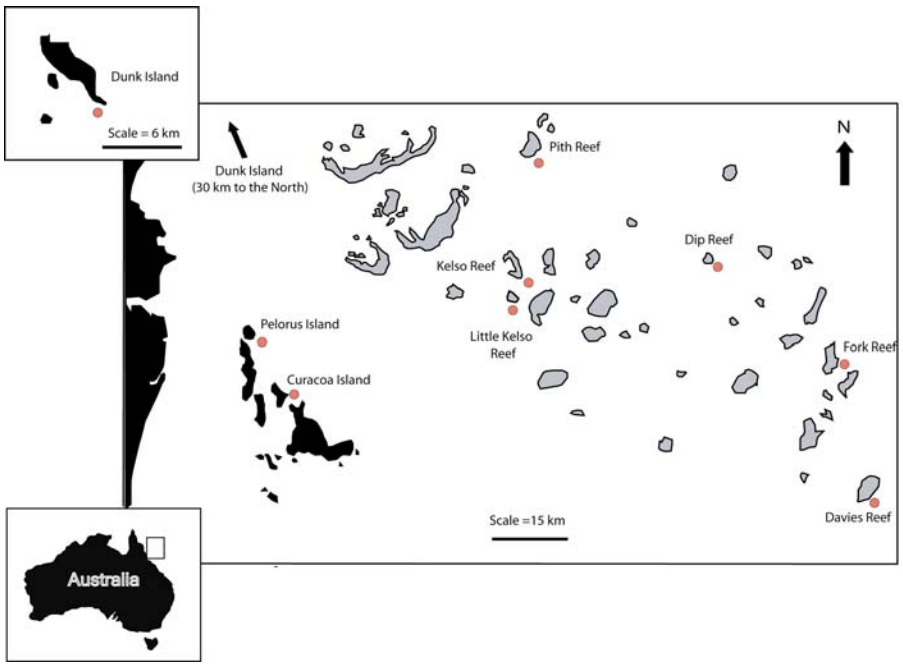

Figure 2.1 Map of reefs surveyed across inner-, mid- and outer-shelf locations on the central GBR, Australia. Red circles indicate reefs surveyed at each shelf location. Black objects represent land and grey objects represent reef complexes.

2.2.2 Distribution and abundance

To quantify the cross-shelf distribution and abundance patterns of *R. odorabile*, 250 m^2 (25 m x 10 m) belt transects were surveyed at each shelf location (inner-, mid- and outer-shelf) using a structured hierarchical sampling design (Figure 2.2). At each shelf location, three reefs (separated by 10s km) of similar habitat structure were surveyed. At each of these reefs, three sites (separated by 100 to 200 m) were surveyed. At each site, the abundance of *R. odorabile* was quantified at depths between 5 and 15 m, using five 250 m^2 belt transects that were randomly laid and did not overlap. The depth of each individual sponge was recorded during surveys to examine the relationship between depth and abundance of *R. odorabile*. Surveys were limited to between 5 and 15 m as the abundance of sponges above 5 m on the central GBR is minimal and the fringing reefs terminate at 15 m on inner-shelf reefs (Wilkinson and Cheshire 1989; Bannister et al. 2007).

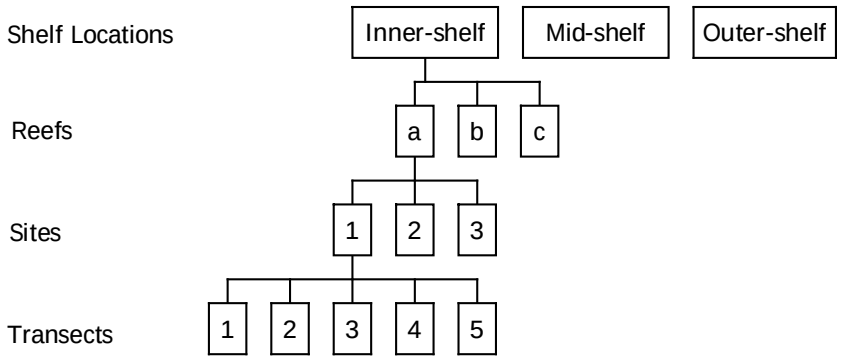

Figure 2.2 Hierarchical sampling design employed to determine cross shelf distribution, abundance and size patterns of *R. odorabile* on the central GBR, Australia.

2.2.3 Size and size frequency distributions

To examine the mean size and size frequency distributions of *R. odorabile* across the three shelf locations in a non destructive manner, the greatest dimensions for length, width and height were

measured to the nearest half cm to determine volume measurements for every individual *R. odorabile* recorded in the distribution and abundance surveys. There is a high correlation between volume estimates and actual biomass (Duckworth and Battershill 2003). Size was measured using a ruler attached to the diver's slate and was reported as volume (cm^3). Specimens are grouped into four size classes: <600 cm^3 (small), 600 - 1700 cm^3 (medium), 1700 - 4300 cm^3 (large) and >4300 cm^3 (very large). These size classes were chosen as they are the four quartiles for all *R. odorabile* measured across the three shelf locations. The relationship between the size of *R. odorabile* and depth was also examined.

2.2.4 Statistical analysis

All statistical analyses were performed using SYSTAT version 10. To ensure data met the assumptions of the statistical analyses performed all data were checked for homogeneity of variance and normality using standardized residuals versus predicted values plots and Q-Q plots of residuals, respectively. Data transformations for each statistical analysis are discussed below. The measure of variation associated with the reporting of all mean values throughout this thesis is 1 standard error (SE).

To determine differences in (a) the mean abundance and (b) the mean size of *R. odorabile* across each shelf location, the mean abundance and the mean size data for *R. odorabile* was analysed separately using 3-factor nested analysis of variance (ANOVA) models (Factors: (1) Shelf (fixed factor) – 3 shelf locations, (2) Reef nested within Shelf (random factor) – 3 reefs per shelf, Site nested within Reef (random factor) – 3 sites per reef). Abundance data and size data were square root transformed to meet the assumptions of ANOVA (Underwood 1981). Statistical differences were interpreted using Tukey's honestly significant difference (HSD) multiple comparison test (Quinn and Keough 2002).

To determine whether depth influences the size of *R. odorabile* across each shelf location, records of individual sizes of *R. odorabile* collected during the cross shelf distribution and abundance surveys were pooled into a single sample for each shelf location. Simple linear correlations were used to examine the relationship between the size of *R. odorabile* and the depth they inhabit at inner-, mid- and outer-shelf locations. Relationships between size and depth were interpreted using Pearson correlation coefficients.

Size frequency distribution data was used to determine differences in (a) the percentage and (b) the number of *R. odorabile* within each size class for each shelf location (inner-, mid- and outer-shelf). The percentage and number of *R. odorabile* within each size class was analysed separately using 2-factor nested ANOVA models for each size class (<600 cm^3, 600 - 1700 cm^3, 1700 - 4300 cm^3, >4300 cm^3) (Factors: (1) Shelf (fixed factor) – 3 shelf locations and (2) Reef nested within Shelf (random factor) – 3 reefs per shelf location). Percentage data for <600 cm^3 size class was arcsin square root transformed and data for the number of *R. odorabile* within the <600 cm^3 and >4300 size classes were log and log (x +1) transformed, respectively, to meet the assumptions of ANOVA (Underwood 1981). Statistical differences were interpreted using Tukey's HSD multiple comparisons tests (Quinn and Keough 2002). Due to multiple testing a Bonferroni transformed experiment-wise $\alpha = 0.0125$ was employed to reduce Type 1 error rates (Quinn and Keough 2002).

2.3 Results

2.3.1 Abundance of *Rhopaloeides odorabile*

R. odorabile was present at all 27 sites surveyed across the central GBR, with a total of 2043 individuals recorded. The abundance of *R. odorabile* varied significantly between shelf locations with the mean abundance on mid- (20.3 ± 2.2 individuals.250 m^{-2}) and outer-shelf reefs (22.6 ± 6.4

individuals.250 m^{-2}) being 3.5 times greater than inner-shelf reefs (6.2 ± 2.4 individuals.250 m^{-2}) (Figure 2.3, Table 2.1). The abundance of *R. odorabile* also differed significantly between reefs within shelf locations with the highest abundance recorded at Pith Reef on the outer-shelf reef (35.2 ± 3.3 individuals.250 m^{-2}), whilst the lowest abundance was recorded at Dunk Island on the inner-shelf reef (1.6 ± 0.12 individuals.250 m^{-2}) (Figure 2.4).

The distribution of *R. odorabile* on inner-shelf fringing reefs was restricted to depths <10 m with a preference to inhabit shallower depths (Figure 2.5). However, the abundance of *R. odorabile* on mid- and outer-shelf reefs has a Gaussian distribution inhabiting the reef substrate between 5 and 15 m. The depths of 9.5 and 10 m have the most individuals on both mid- and outer-shelf reefs, respectively (Figure 2.5).

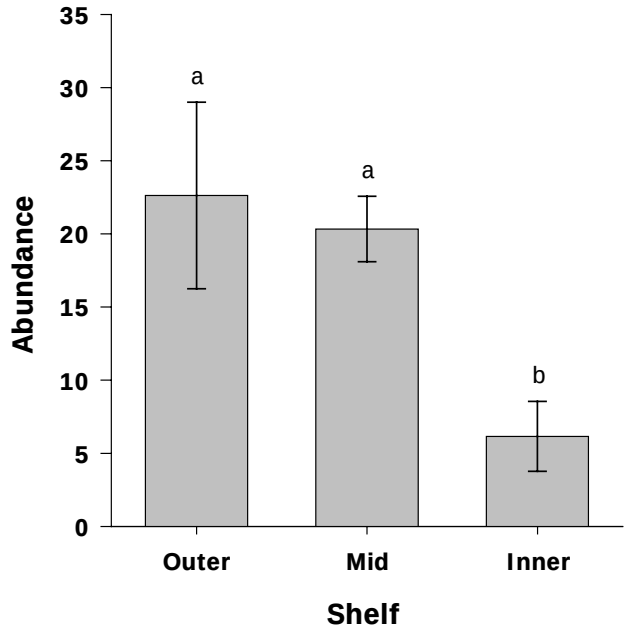

Figure 2.3 Mean abundance (± SE) of *R. odorabile*.250 m^{-2} across inner-, mid- and outer-shelf locations on the central GBR, Australia. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05 for square root transformed data, n=3 per shelf location).

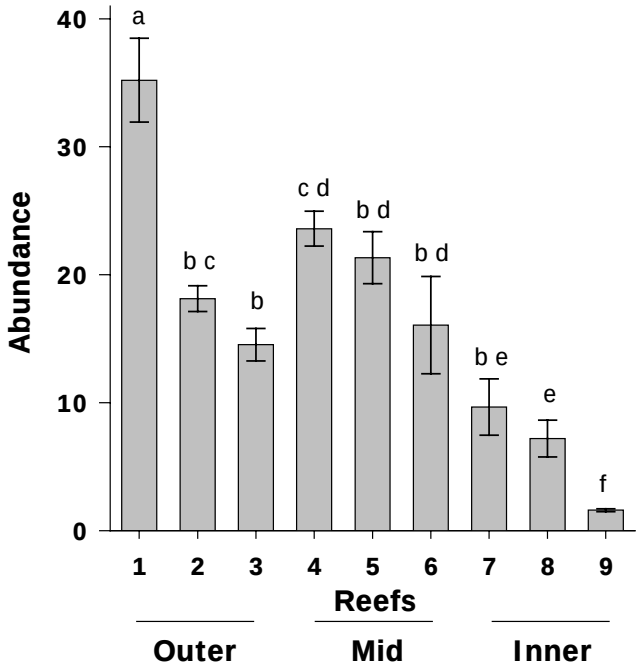

Figure 2.4: Mean abundance (± SE) of *R. odorabile*.250 m^{-2} at different reefs across inner-, mid- and outer-shelf locations on the central GBR, Australia. 1 – Pith Reef, 2 – Dip Reef, 3 – Fork Reef, 4 – Davies Reef, 5 – Kelso Reef, 6 – Small Kelso Reef, 7 – Curacoa Island, 8 – Pelorus Island and 9 – Dunk Island. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05 for square root transformed data, n=3 per reef).

Table 2.1 Three-factor nested ANOVA model for square root transformed mean abundance of *R. odorabile*.250 m^{-2} across inner-, mid- and outer-shelf locations of the central GBR, Australia.

Source	**df**	**MS**	**F**	***P***
Shelf	2	81.547	5.98	0.037
Reef (Shelf)	6	13.635	11.98	0.000
Site (Reef)	18	1.139	1.52	0.098
Error	108	0.751		

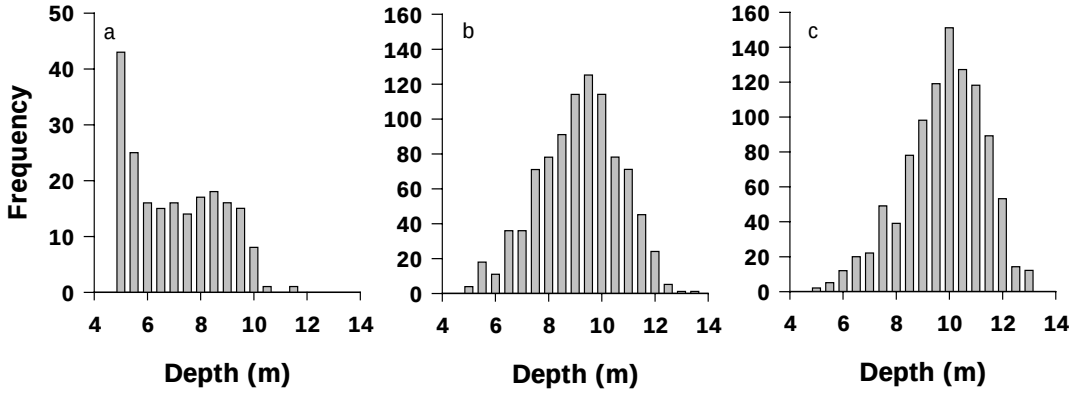

Figure 2.5 Depth frequency histogram of *R. odorabile* across (a) inner-shelf, (b) mid-shelf and (c) outer-shelf locations on the central GBR, Australia.

2.3.2 Size of *Rhopaloeides odorabile*

The mean size (volume) of *R. odorabile* did not differ significantly across shelf locations (Figure 2.6, Table 2.2) with mean sizes of 3096.3 ± 1394.2 cm^3, 2323.8 ± 548.3 and 4236.3 ± 941.9 cm^3, across inner-, mid- and outer-shelf reefs, respectively. The low variation in mean size of *R. odorabile* between sites within reefs, suggests that the high variation observed across shelf locations is being driven by natural variation in the mean size of *R. odorabile* both within and between shelf locations (Table 2.2). The mean size (volume) of *R.odorabile* was significantly different between reefs with the largest mean size at Pith Reef (5722.6 ± 394.2 cm^3) and the smallest mean size at Dunk Island (766.6 ± 249.5 cm^3) (Figure 2.7). The largest individuals recorded across shelf locations were approximately 76000 cm^3 at Pith Reef (outer-shelf reef), 46000 cm^3 at Small Kelso Reef (mid-shelf reef) and 45000 cm^3 at Curacoa Island (inner-shelf reef).

Furthermore, non-significant linear correlations demonstrated that the size of *R. odorabile* across inner- ($r^2 = 0.006$, $P = 0.079$) and outer-shelf reef locations ($r^2 = 0.003$, $P = 0.056$) was not restricted by the depth at which they grew (Figure 2.8). On mid-shelf reefs, the size of *R. odorabile* decreased

significantly with increasing depth ($r^2 = 0.014$, $P < 0.001$), however, the low r^2 value indicated that this relationship was very weak.

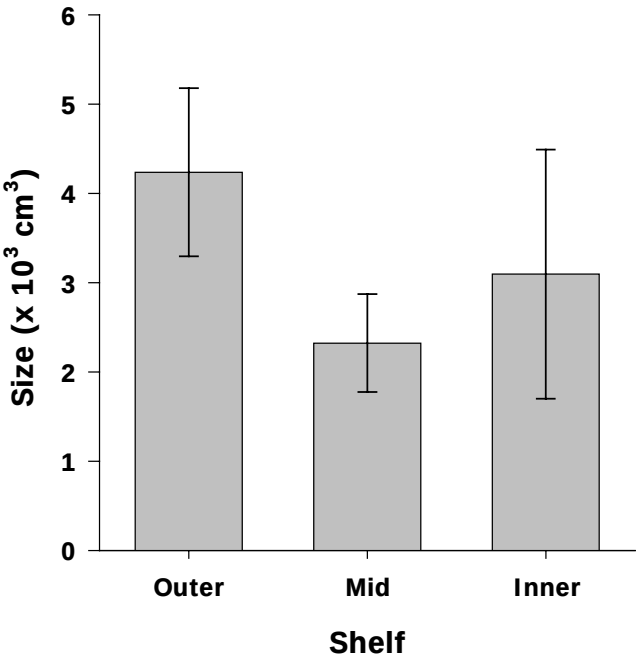

Figure 2.6 Mean size ($x10^3$ cm^3, ± SE) of *R. odorabile* across inner-, mid- and outer-shelf locations on the central GBR, Australia (n=3 per shelf location).

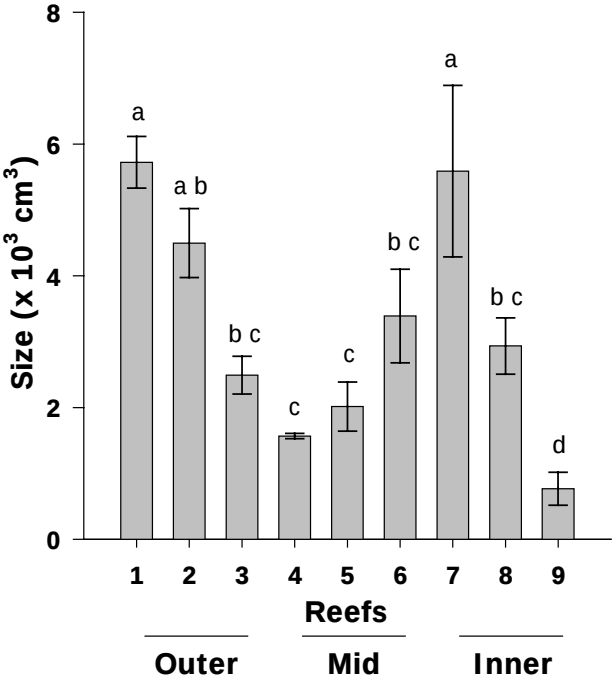

Figure 2.7 Mean size (x10^3 cm^3, ± SE) of *R. odorabile* at different reefs across inner-, mid- and outer-shelf locations on the central GBR, Australia. 1 – Pith Reef, 2 – Dip Reef, 3 – Fork Reef, 4 – Davies Reef, 5 – Kelso Reef, 6 – Small Kelso Reef, 7 – Curacoa Island, 8 – Pelorus Island and 9 – Dunk Island. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05 for square root transformed data, n=3 per reef).

Table 2.2 Three-factor nested ANOVA model for square root transformed mean size (cm^3) of *R. odorabile* across inner-, mid- and outer-shelf locations of the central GBR, Australia.

Source	df	MS	F	*P*
Shelf	2	4121.219	0.91	0.451
Reef (Shelf)	6	4522.159	14.81	0.000
Site (Reef)	18	305.264	1.59	0.075
Error	108	191.997		

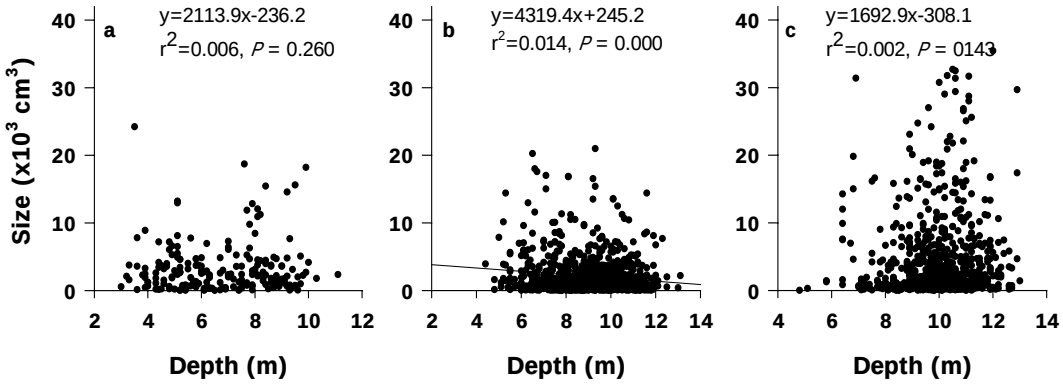

Figure 2.8 Linear correlation between size (x10^3 cm^3) and depth (m) of *R. odorabile* surveyed across (a) inner-shelf, (b) mid-shelf and (c) outer-shelf locations of the central GBR, Australia.

2.3.3 Size structure of *Rhopaloeides odorabile* populations

Across the central GBR, minimal variation exists within the population structure of *R. odorabile* when assessed within four size classes across shelf locations. The mean number of individuals within the two smaller size classes (<600 cm^3 and the 600 – 1700 cm^3) differed significantly across shelf locations (Figure 2.9a, Table 2.3a). Mid- and outer-shelf reefs had 6.7 and 4.5 times more individuals <600 cm^3, respectively, than inner-shelf reefs. In addition, mid- and outer-shelf reefs had 6.5 and 5.1 times more individuals within the 600 – 1700 cm^3 size class, respectively, than inner-shelf reefs (Figure 2.9a). Furthermore, significant variation between reefs within shelf locations occurs for the number of individuals within the two larger size classes (1700 – 4300 cm^3 and >4300 cm^3) (Table 2.3a). Despite between shelf variations in the number of *R. odorabile* individuals within each size class, *R. odorabile* populations across inner-, mid- and outer-shelf locations had similar percentages of individuals within each of the four size classes (Figure 2.9b, Table 2.3b). However, there is significant variation between reefs within shelf locations for the percentage of *R. odorabile* individuals <600 cm^3 and >4300 cm^3 (Table 2.3b).

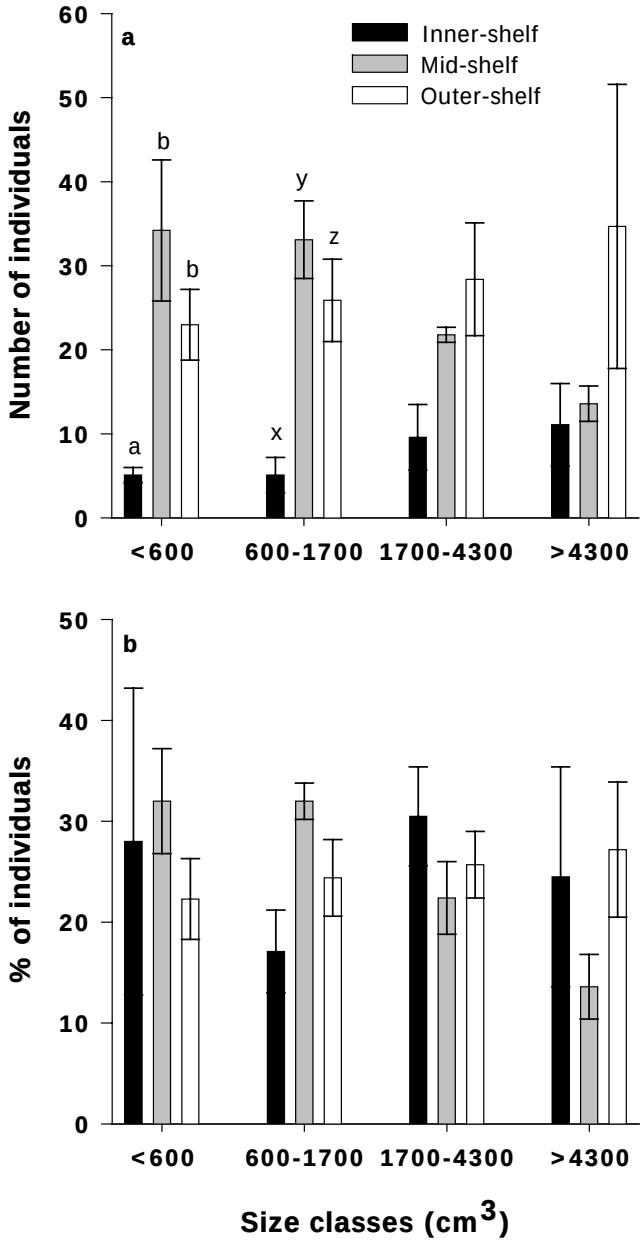

Figure 2.9 (a) Mean number (± SE) and (b) mean percentage (± SE) of *R. odorabile* between four size classes (<600 cm^3 600 - 1700 cm^3, 1700 - 4300 cm^3 and >4300 cm^3) across inner-, mid- and outer-shelf locations on the central GBR, Australia. Superscripts denote significant differences within each size class (Tukey's HSD multiple comparison test, P <0.05, n=3 for each shelf location).

Table 2.3 Two-factor nested ANOVA models comparing the variation in (a) the mean number and (b) the mean percentage of *R. odorabile* between four size classes (<600 cm^3 600 - 1700 cm^3, 1700 - 4300 cm^3 and >4300 cm^3) across inner-, mid- and outer-shelf locations of the central GBR, Australia. Bonferroni corrected $\alpha = 0.05/4 = 0.0125$

Size class		**<600 cm^3**			**600 – 1700 cm^3**			**1700 – 4300 cm^3**			**>4300 cm^3**		
Source	**df**	**MS**	**F**	***P***	**MS**	**F**	***P***	**MS**	**F**	***P***	**MS**	**F**	***P***
(a)													
Shelf	2	10.403	20.70	0.002	1901.815	12.88	0.007	825.926	4.53	0.063	5.135	2.21	0.190
Reef (Shelf)	6	0.503	1.78	0.160	147.667	3.422	0.020	182.333	5.03	0.003	2.319	11.58	0.000
Error	18	0.283			43.148			36.222			0.200		
(b)													
Shelf	2	0.029	0.27	0.774	0.050	4.87	0.055	0.015	1.03	0.412	0.047	0.89	0.458
Reef (Shelf)	6	0.110	12.95	0.000	0.010	2.25	0.086	0.014	3.11	0.029	0.053	9.77	0.000
Error	18	0.008			0.005			0.005			0.005		

2.4 Discussion

The key findings for this study are that the mean abundance of *R. odorabile* across shelf locations of the central GBR increased significantly with increasing distance from the coastline. Despite significant differences in the abundance of *R. odorabile* across shelf locations, there were no statistical differences in the mean size of individuals at the same spatial scale. Furthermore, the size structure of *R. odorabile* populations across inner-, mid- and outer-shelf locations were similar, despite *R. odorabile* being up to 3.5 times more abundant on mid- and outer-shelf locations compared to inner-shelf locations. There was a non-consistent depth effect with *R. odorabile* on inner-shelf locations being more abundant in shallow habitats (at approximately 5 to 8 m) compared to mid- and outer-shelf locations, where *R. odorabile* were more abundant in deeper habitats (at approximately 10 m). These distinct differences in the abundance and depth distribution of *R. odorabile* across inner-, mid- and outer-shelf locations appear to be related to the persistent environmental gradients that are present across shelf locations of the central GBR (Devlin and Brodie 2005).

The reduced abundance of *R. odorabile* on inner- compared to mid- and outer-shelf reefs contradicts an earlier study by Wilkinson and Cheshire (1989), where they found that *R. odorabile* was slightly more abundant on inner shelf reefs than mid- and outer-shelf reefs. However, the depth distribution patterns of *R. odorabile* on mid-shelf reefs identified during this study are similar to those identified by Wilkinson and Evans (1989), where the abundance of *R. odorabile* increases between 7 to 10 m. This begs the question as to the potential impacts that may have affected distribution and abundance patterns over time, with what appears to be a significant impact on historical distributions on inshore reefs. Due to the different sampling efforts carried out (i.e. transect size, reefs surveyed and area covered) in this study and the study conducted by Wilkinson and Cheshire (1989), together with high patchy distributions (Bannister et al. 2007) and high

variability in the abundance of *R. odorabile* between reefs sampled (Figure 2.4), direct comparisons between studies would be invalid. However, given the twenty year period between studies and the difference in the abundance of *R. odorabile* on inshore reefs it is reasonable to hypothesise that declining water quality in inshore coastal habitats of the central GBR, including reduced light availability associated with increased sedimentation and nutrients (McCulloch 2003; Udy et al. 2005) may have affected benthic community structure over time. Therefore, abundances and depth distributions patterns of *R. odorabile* across shelf locations of the central GBR may be explained by these declining water quality parameters; this being the focus of the remainder of this thesis.

Light availability (Wilkinson and Vacelet 1979; Jokiel 1980; Wilkinson and Trott 1985) may explain differences in abundance and depth distribution of *R. odorabile* across shelf locations. Reduced light levels associated with increased turbidity and nutrients on inner-shelf reefs (Anthony et al. 2004) negatively impacts coral reef ecosystems (Fabricius 2005). Reduced light availability leads to decreased photosynthesis by zooxanthallae, resulting in reduced energy acquisition and slower growth of corals (Rogers 1979; Telesnicki and Goldberg 1995; Anthony and Hoegh-Guldberg 2003). The response of sponges to reduced light levels is similar to corals, where growth is compromised at lower levels of energy acquisition (Wilkinson and Vacelet 1979; Thacker 2005). As such, sponges possessing photosymbionts generally live on mid- and outer-shelf reefs where light availability is not compromised by increased turbidity and nutrients (Wilkinson and Trott 1985; Wilkinson and Cheshire 1989, Cleary et al. 2005). However, some phototrophic species have been recorded on inner-shelf reefs (Bannister et al. 2007), suggesting that if *R. odorabile* relies on energy acquisition from photo-symbionts, light attenuation on inner-shelf reefs may explain the shallower depth colonisation by *R. odorabile* compared to mid- and outer-shelf reefs. However, a critical first step in examining this hypothesis is to determine if *R. odorabile* is a phototrophic sponge and what components of energy requirements are obtained through photosymbionts.

There may also be physiological regulation of settlement and growth of *R. odorabile* at specific depths on mid- and outer-shelf reefs. If *R. odorabile* colonise shallower depths their surface tissues may be exposed to excess UV radiation, resulting in tissue damage and death (Jokiel 1980; Regoli et al. 2000). However, if *R. odorabile* colonise greater depths, energy obtained from photosymbionts may be reduced due to reductions in photosynthetic activity (Cheshire and Wilkinson 1991). Although *R. odorabile* prefers to inhabit substrate at deeper depths on mid- and outer-shelf reefs, there is no relationship between depth and sponge size, suggesting that depth may not influence the growth of *R. odorabile*. However, light does influence larval settlement and recruitment of *R. odorabile* with larvae displaying positive phototactic behaviour up to 24 h after release (Whalan et al. in press). Therefore, to determine an impact of light availability on the distribution and abundance of *R. odorabile* it is critical to establish whether *R. odorabile* possess photosymbionts. If present, the influence of light aiding energy acquisition and its role in determining habitat preferences and the distribution of *R. odorabile* across reef shelf locations needs to be established.

Despite few studies detailing the impacts of environmental gradients on sponges in coral reef habitats, differences in the abundance of *R. odorabile* across inner-, mid- and outer-shelf reef locations may also be explained by changes in sedimentation associated with environmental gradients across shelf locations (Devlin and Brodie 2005). In hard benthic habitats sedimentation influences the structure, biomass and metabolism of marine benthic communities (Balata et al. 2005) and is a key environmental factor influencing the distribution, abundance and species richness of sponge assemblages across temperate and tropical reef habitats (Zea 1994; Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006). The exposure of *R. odorabile* on inner-shelf reefs to fine terrigenous sediments and high sedimentation rates (Larcombe and Woolfe 1999) may have multiple and/or cascading effects that may ultimately reduce the abundance of individuals. Sedimentation may result in the smothering, burial and/or scouring of newly settled recruits and

adult sponges (Ilan and Abelson 1995), which may cause death and/or remove individuals from the substratum (Irving and Connell 2002; Airoldi 2003; Maldonado et al. 2008). To prevent smothering and burial some sponges are capable of gross morphological adaptation (McDonald et al. 2002; Bell 2004) while others produce mucus sheaths (Turon et al. 1999) and actively creep away from sites of high sedimentation (Maldonado and Uriz 1999). Furthermore, high concentrations of fine suspended sediments may also clog inhalant canals and filtering structures of sponges resulting in reduced pumping activity (Gerrodette and Flechsig 1979) and reduced clearance rates of sponges (Lohrer et al. 2006). In addition, clogged inhalant canals and filtering structures may reduce the retention efficiency of ultraplankton, however, this remains to be examined for sponges.

Morphological and/or physiological adaptations to increased sedimentation may impose energetic constraints arising from reduced energy intake and/or an increase in energy output (Coma and Ribes 2003). These energetic constraints may result in the reduced reproductive output of sponges (Roberts et al. 2006; Whalan et al. 2007a), which may subsequently result in reduced recruitment of larvae, and consequently reduce the abundance of individuals. Although sedimentation has been proposed to influence the structure of sponge assemblages (Zea 1994; Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006), there is a dearth of information examining the effects of suspended sediment grain size and mineralogical composition on sponge physiology and ecology. This is despite a plethora of research identifying the impacts of sediment grain size and mineralogical composition of suspended sediments on coral physiology and ecology (reviewed in Fabricius 2005; Weber et al. 2006). Therefore, before drawing conclusions on the impacts of sedimentation on the distribution and abundance of *R. odorabile* across shelf locations of the central GBR it is important to characterise suspended sediment grain size and mineralogy within sponge habitats across these shelf locations, and to relate these characteristics to the abundance and distribution of *R. odorabile*.

Distinct differences in cross shelf phytoplankton composition, biomass and community dynamics across shelf locations of the central GBR (Revelante et al 1982; Furnas and Mitchell 1986) may also be responsible for differences in the abundance and distribution of *R. odorabile* within and across shelf locations. Food availability is of critical importance to the growth, reproduction and dynamics of marine benthic invertebrates (reviewed in Coma and Ribes 2003). While food availability has been suggested as a key factor influencing the structure of sponge communities (Storr 1976; Wilkinson and Cheshire 1989;Tanaka 2002; Lesser 2006) few studies have investigated food availability as a constraining factor for marine sponges in coral reef habitats. Recent studies by Lesser (2006) and Trussell et al. (2006) have demonstrated that food availability is a critical factor affecting the growth and subsequently the distribution and abundance of marine sponges in coral reef habitats. Lesser (2006) identified that the growth rate and subsequent distribution and abundance of three coral reef sponges *Callyspongia vaginalis*, *Agelas conifera* and *Aplysina fistularis* are driven by food availability. Trussell et al. (2006) also concluded that increased abundance and growth rates of the coral reef sponge, *Callyspongia vaginalis* is linked to increased food availability. Therefore, to determine an impact of food availability on the distribution and abundance of *R. odorabile* it is necessary to quantify food availability within the habitat of *R. odorabile* across shelf locations of the central GBR and subsequently determine its retention within these habitats.

In conclusion, light, sedimentation and food availability, key factors associated with environmental gradients across shelf locations of the GBR, may help explain the abundance and distribution patterns of *R. odorabile* identified across inner-, mid- and outer-shelf reef locations. However, it is impossible to conclude that differences in the abundance and distribution of *R. odorabile* are linked only to these environmental factors. Other biotic and abiotic factors may also contribute to variation in their abundance and distribution, including predation and competition (reviewed in Wulff 2006), recruitment (Zea 1993; Maldonado and Young 1996, 1998), substrate type and angle (Bell and

Barnes 2000a,b), exposure level (Wilkinson and Evans 1989; Bannister et al. 2007) and pollution (Cebrian and Uriz 2007a,b). However, given the strong supportive evidence of the impacts of light and sediment on the distribution, abundance and physiology of sponges, the remainder of this thesis will focus on understanding the role of light and sediment in structuring populations of *R. odorabile* across reef shelf locations. Specifically, Chapter 3 will investigate the photo-physiological potential of *R. odorabile*; Chapter 4 will characterise the structure of suspended sediment within sponge habitats, including grain size and mineralogical composition. Chapter 5 will quantify spatial and temporal variations in the retention efficiencies of ultraplankton by *R. odorabile*; and Chapter 6 will, for the first time, measure the respiratory response of sponges to contrasting mineralogical suspended sediments.

Chapter 3 – Photophysiology of *Rhopaloeides odorabile*

3.1 Introduction

Animal-plant and animal-microbial symbioses are very common in clear oligotrophic waters surrounding coral reefs. The most prevalent of these symbioses are between hard corals (coelenterates) and zooxanthallae (dinoflagellates) (Smith and Douglas 1987) which provide corals with up to 98% of their carbon requirements photosynthetically (Muscatine 1990). The maintenance, growth and diversity of coral reefs are heavily reliant on coral symbioses through the processes of photosynthesis, mineral recycling and the production of carbonate skeletons (Smith and Douglas 1987).

Like corals, many species of sponges form symbioses with marine algae including filamentous cyanobacteria, zooxanthellae, diatoms and other unicellular algae (reviewed by Sara et al. 1998). In addition, sponges have highly specific but complex interactions with a consortium of bacteria, comprising of up to 18 different bacterial and archaeal phyla, which can contribute up to 40% of the sponge biomass (Taylor et al. 2007b; reviewed in Taylor et al. 2007a). Diverse microbial associations in sponges contribute to their nutrition, including the assimilation of dissolved organic carbon and nitrogen (DOC, DON) and photosynthetically derived carbon (reviewed in Taylor et al. 2007a). Cyanobacteria are the most common symbionts with sponges on the GBR (Wilkinson 1978) providing phototrophic sponges with at least 50% of their nutrition as fixed carbon from photosynthesis (Wilkinson 1983; Wilkinson and Trott 1985). Other benefits of sponge-microbe associations include nitrification, methane oxidation, sulfate reduction and dehalogenation processes (reviewed in Taylor et al. 2007a).

On the GBR, the majority of sponges found on inner-shelf reefs are heterotrophic, whilst those found on outer-shelf reefs are predominantly phototrophic or mixotrophic (Wilkinson and Trott 1985; Wilkinson 1987; Wilkinson and Cheshire 1989). The dominance of heterotrophic sponges on inner-shelf reefs is suggested to be due to increased suspended sediments, nutrients and reduced light availability associated with land-based runoff from adjacent rivers and streams (Wilkinson and Cheshire 1989). In contrast, clear water drives the dominance of phototrophic sponges on outer-shelf reefs due to the higher light intensities required for growth and survival (Wilkinson and Trott 1985).

Some sponge species on the GBR colonise inner-, mid- and outer-shelf reefs (Wilkinson and Cheshire 1989) suggesting their adaptation to multiple environments. One such species, *R. odorabile,* colonises both inshore and offshore coral reef habitats (see Chapter 2 and Wilkinson and Cheshire 1989) with distinct colour morphs between locations. *R. odorabile* inhabiting inshore

reefs are slender and show partial surface depigmentation (Figure 3.1a), whereas on offshore reefs *R. odorabile* are massive and have dark reddish-brown surface pigmentation (Figure 3.1b). This pigmentation is characteristic of sponges possessing symbiotic cyanobacteria (Giano et al. 1977; Wilkinson 1980) and is assumed to confirm phototrophism. However, surface pigmentation and/or the presence of cyanobacterial symbionts should not infer phototrophic nutrition in sponges. Wilkinson (1983) identified that only six of nine species of sponges possessing cyanobacterial symbionts on the GBR were net primary producers. This highlights the importance of modelling photosynthetic production using oxygen evolution experiments to quantify autotrophic contribution to sponge nutrition.

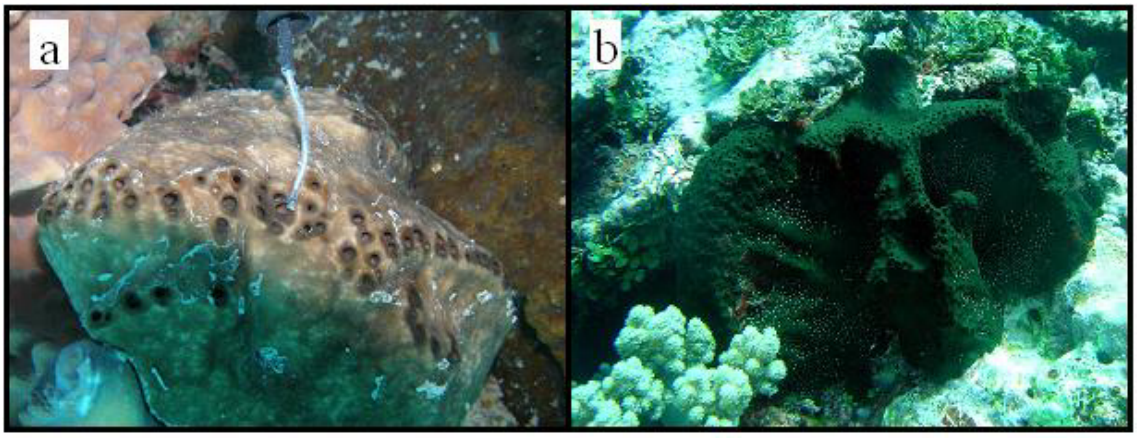

Figure 3.1 (a) An inner-shelf *R. odorabile*, displaying a characteristic slender shape and partial surface depigmentation and (b) An outer-shelf *R. odorabile*, displaying a massive form with characteristic dark reddish-brown surface pigmentation.

Recent research has isolated and identified the culturable microbial community of *R. odorabile*. This community includes a unique cyanobacterium related to the genera *Leptolyngbya* and *Plectonema* (Webster and Hill 2001). The isolated cyanobacteria from *R. odorabile* could not be enumerated and its role within *R. odorabile* remains unclear (Webster and Hill 2001). However, the distribution of *R. odorabile* on exposed fore reef slopes at depths between 5 and 15 m on mid- and outer-shelf reefs and <10 m on inner-shelf reefs (see Chapter 2) suggests that a symbiotic

relationship with this unique cyanobacterium may restrict the depth distribution of *R. odorabile*. This restricted depth distribution may also coincide with the transfer of energy to *R. odorabile* through the process of photosynthesis.

Therefore, this chapter aims to determine the role of light, by way of photosynthesis, as a factor affecting the distribution and abundance of *R. odorabile* across the continental shelf of the central GBR, as described in Chapter 2. It specifically addresses the following: (1) Is *R. odorabile* phototrophic? (2) Does the phototrophic component of *R. odorabile* vary between inner-, mid- and outer-shelf reef locations?

3.2 Materials and methods

3.2.1 Collection of specimens

In January 2005, ten sponge explants (one explant per sponge) were cut from ten *R. odorabile* individuals at Pith Reef (outer-shelf reef), Rib Reef (mid-shelf reef) and Pelorus Island (inner-shelf reef). Five sponge explants (one explant per sponge) were also cut from five *Carteriospongia foliascens* individuals (a known phototrophic sponge, Bergquist et al. 1988) at Rib Reef to use as a positive control for photophysiology experiments. At each location these explants were placed into plastic moulded recovery cages (Aqua-Tech) that were anchored to the sea floor at 9 m (the same depth that these sponges were collected). Explants were left in these cages for 7 weeks to allow their damaged surfaces to heal and recover (Louden et al. 2007).

Sponge explants were collected from each of the reefs after 7 weeks (March 2005), and placed into 30 l plastic drums with seawater and adequate aeration and transported back to the aquarium facilities at James Cook University (JCU). Sponges were placed into 12 different 30 l aquaria (5 sponges per aquarium) and acclimatised for 3 d prior to conducting respirometry experiments. The

aquaria were maintained in a temperature-controlled laboratory (28°C), reflecting ambient temperatures on the reefs where the explants were collected. Each aquarium had a mesh rack mounted on the bottom to maintain adequate water flow around the sponge explants and circulation was provided by a power-head pump (Aqua-clear 402). Seawater in the aquarium system was collected from Cape Cleveland, 50 km south of Townsville and was filtered to 10 μm. Bio-filtration, foam fractionators and sand filters were used to maintain water quality. Each aquarium was illuminated on a 10:14 h light:dark regime.

3.2.2 Photophysiology

To determine if photosynthesises occurs within the tissues of *R. odorabile*, oxygen evolution experiments were carried out using six closed, recirculating transparent perspex flow chambers (2.7 l) fitted with calibrated Clark-type oxygen electrodes (Cheshire Systems, Adelaide). The respirometry system was set up following the methodology from Hoogenboom et al. (2006). Flow chambers provide continuous uni-directional flow to the sponges at approximately 5 to 6 $cm.s^{-1}$ for 25 min intervals. All chambers were flushed with new seawater for 5 min after every 25 min recording interval to maintain oxygen saturation above 85%. Clark-type oxygen electrodes were connected to a multi-sensor signal conditioner, which recorded oxygen concentrations every 30 s onto a central data logger (CR10X Campbell Scientific). The data logger also controlled the flushing cycle (Hoogenboom et al. 2006). Two suspended 400 W metal halide lamps were used to adjust the light intensity over the respirometry chambers, exposing the sponges to 12 discrete light levels for 30 min periods (0, 15, 40, 60, 100, 120, 200, 290, 420, 530, 710 and 900 μmol $photons.m^{-2}.s^{-1}$). Light levels were measured using a Li-192S Li-cor probe connected to a Li-1000 data logger.

Oxygen evolution experiments were run consecutively over 7 days using five sponges per day. Temperature remained constant for all days (27-28°C). On each day of data collection, four

treatment sponge explants (collected from inner-, mid- or outer-shelf treatments) and one control explant (*C. foliascens*) were randomly selected (using a random number table) and placed into the respirometry chambers. One chamber was left empty during each respirometry run to control for photosynthesis and respiration of microorganisms within the water. Chambers were cleaned at the end of each run to prevent biofilm formation (Hoogenboom et al. 2006). Dark respiration was measured for 1 h at the start of the experiment and for the following 6 h period photosynthesis versus irradiance curves (*P-I* curves) were constructed for the sponge explants. Whole explants were weighed immediately following each daily experiment to obtain wet weights, allowing oxygen flux to be compared to literature values (μmol O_2 $g.fw^{-1}.h^{-1}$). Surface tissue samples (1 cm^3) were also collected from each individual sponge explant and frozen at -40°C to quantify photopigments.

3.2.3 Photopigment extraction

To extract photopigments (chlorophyll *a*, c_1, c_2 and phycoerythrin) whole tissue samples were prepared following methods modified from Larkum et al. (1987). To extract water-soluble pigments (phycobiliproteins) tissue samples were frozen and thawed several times in ice-cold buffer (0.2 *M* phosphate pH 7.4). Subsequently, to extract chlorophyll, tissue samples used for phycobiliproteins extractions were freeze dried to remove excess water, then crushed using liquid nitrogen and a mortar and pestle and placed into cold 90% acetone for 24 h. Both acetone and water-soluble extracts were centrifuged at 1000 × *g* to obtain a clear supernatant. The supernatant from each extract was decanted and then scanned separately at wavelengths between 350 and 750 nm using an Agilent 8453 UV-visible spectrophotometer.

3.2.4 Statistical analysis

A hyperbolic tangent model was fitted to photosynthesis-irradiance (*P-I*) data in order to estimate *P-I* curve parameters for net rates of photosynthesis versus irradiance using non-linear estimations (NLE, STATISTICA 1999). A hyperbolic tangent model (Jassby and Platt 1976) was chosen, because it generally provides the best fit (highest r^2 values) to *P-I* data. The *P-I* model used was:

$$P_n = P_{MAX} \tanh(I / I_K) - R_{DARK}$$

where P_n is the hourly rate of photosynthesis (μmol O_2 $g.fw^{-1}.h^{-1}$), R_{DARK} is the rate of respiration in darkness (μmol O_2 $g.fw^{-1}.h^{-1}$), P_{MAX} is the maximum rate of photosynthesis (μmol O_2 $g.fw^{-1}.h^{-1}$), I is the average irradiance (μmol photons $m^{-2}.s^{-1}$) and I_k is the sub-saturation irradiance (μmol photons $m^{-2}.s^{-1}$). This model can only be fitted to the control sponge dataset, as the data for *R. odorabile* from inner-, mid- and outer-shelf reef locations did not fit the model requirements.

To determine whether increased light intensity influences the oxygen evolution potential of *R. odorabile*, simple linear regressions were fitted to the respirometry data to examine the relationship between increased light exposure and the respiration activity of inner-, mid- and outer-shelf reef explants. Furthermore, to examine statistical differences between the mean respiration rates of *R. odorabile* from inner-, mid- and outer-shelf locations, mean respiration data was analysed using a 1-factor ANOVA model. Respiration data were checked for normality and homogeneity of variance using frequency histograms of residuals and plots of residuals versus means, respectively. Statistical differences were interpreted using Tukey's HSD multiple comparison test (Quinn and Keough 2002).

3.3 Results

3.3.1 Photophysiology

All explants of *R. odorabile* did not have a *P-I* response to increasing light intensity (Figure 3.2), demonstrating unequivocally that photo-symbionts are not present in the tissues of *R. odorabile*. However, the *P-I* response for the control sponge *C. foliascens* was very precise. *C. foliascens* had a strong fit to the hyperbolic tangent model indicated by $r^2 > 0.98$ (Figure 3.2). Also, the standard errors of I_K and P_{MAX} for *C. foliascens* ranged between 5% and 10% of their parameter estimates only (Table 3.1). These results confirm that the respirometry equipment was working correctly during experimental runs.

The mean rate of respiration of *R. odorabile* from inner- ($r^2 = 0.11$, n = 10, $P = 0.274$), mid- ($r^2 = 0.28$, n = 10, $P = 0.060$) and outer-shelf locations ($r^2 = 0.18$, n = 10, $P = 0.154$) did not differ significantly with increasing light exposure (0 - 900 μmol photons $m^{-2}.s^{-1}$). However, the rate of respiration between *R. odorabile* from different shelf locations differed significantly ($F_{2,27} = 4.459$, $P = 0.021$) with the mean respiration rate of *R. odorabile* from inner-shelf locations (1.99 ± 0.14 μmol O_2 $g.fw^{-1}.h^{-1}$) being significantly lower than *R. odorabile* from mid- and outer-shelf locations (2.46 ± 0.12 μmol O_2 $g.fw^{-1}.h^{-1}$). However, mean respiration rates of *R. odorabile* from mid-shelf locations (2.50 ± 0.15 μmol O_2 $g.fw^{-1}.h^{-1}$) did not differ significantly than *R. odorabile* from outer-shelf locations (Figure 3.3).

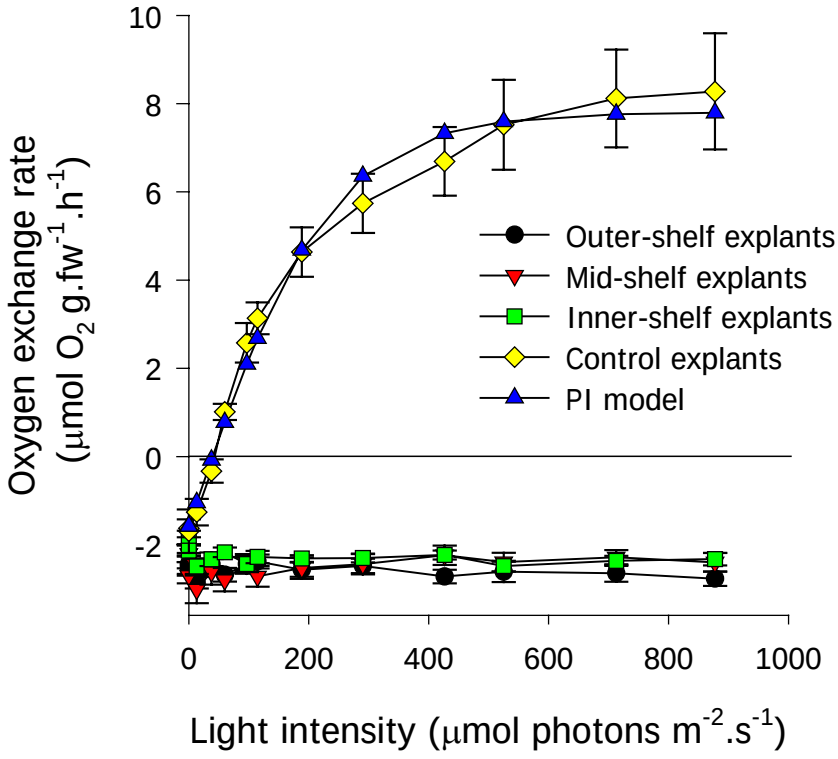

Figure 3.2 *P-I* curves for *R. odorabile* from inner-, mid- and outer-shelf locations and the control sponge *C. foliascens*. The fitted *P-I* model has a similar shape to the *P-I* curve for *C. foliascens*, indicating its strong fit.

Table 3.1 Results of non-linear estimations of steady state sub-saturation irradiance (I_K) and steady state maximum rate of gross photosynthesis (P_{MAX}) for the control sponge *C. foliascens*.

	Estimate	Standard error	*P*
P_{MAX} (μmol O_2 g.fw^{-1}.h^{-1})	9.386	0.304	<0.001
I_K (μmol photons m^{-2}.s^{-1})	233.691	12.447	<0.001
R_{DARK} (μmol O_2 g.fw^{-1}.h^{-1})	1.582	7.012	<0.001

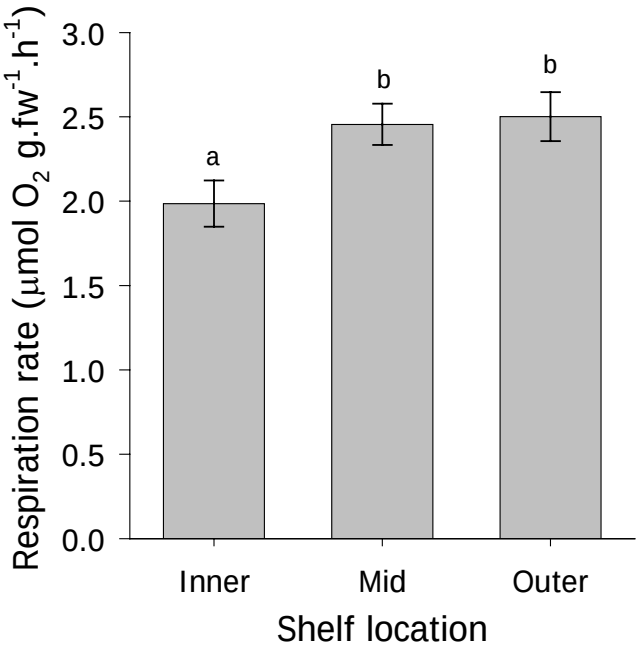

Figure 3.3 Mean respiration rate (µmol O_2 g.fw^{-1}.h^{-1}, ± SE) of *R. odorabile* from inner-, mid- and outer-shelf locations. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05, n=5 per shelf location).

3.3.2 Photopigment extraction

Photopigment analysis of tissues from inner-, mid- and outer-shelf explants of *R. odorabile* demonstrated there are no photo-pigments present within the upper 1 cm^3 of sponge tissue (Figures 3.4 and 3.5). This was the case for both the water-soluble red and blue phycobiliproteins (500 - 600 nm and 550 - 560 nm, respectively) and also for the acetone extracted chlorophylls (630 and 664 nm). However, both red phycobiliproteins and chlorophyll photopigments were present in the tissue of the control sponge *C. foliascens* (Figures 3.4 and 3.5).

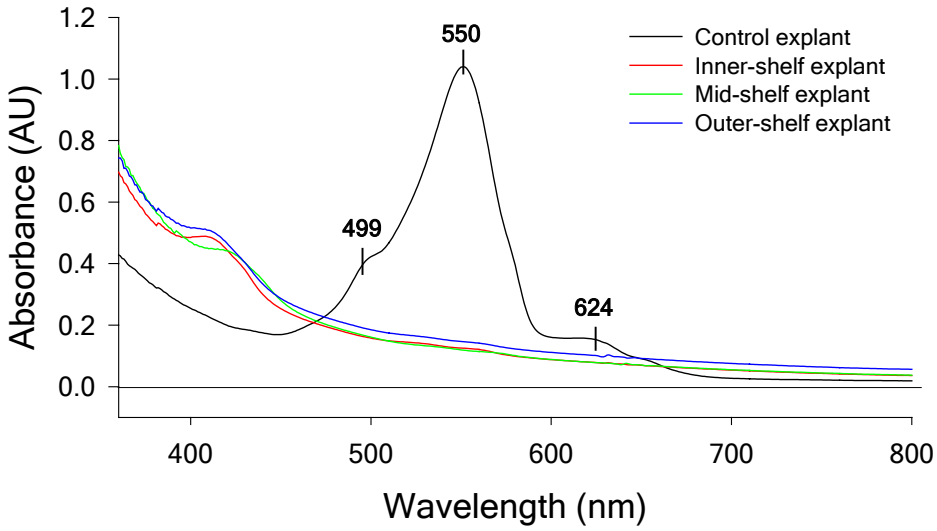

Figure 3.4 Mean absorbance spectra of water-soluble pigments for inner- (n=10), mid- (n=10) and outer-shelf reef explants (n=10) of *R. odorabile* and for explants of *C. foliascens* (n=5). Pigments expressed between 500 and 600 nm wavelength are characteristically red phycobiliproteins (Phycoerythrin, Jeffery and Hallegraff 1990).

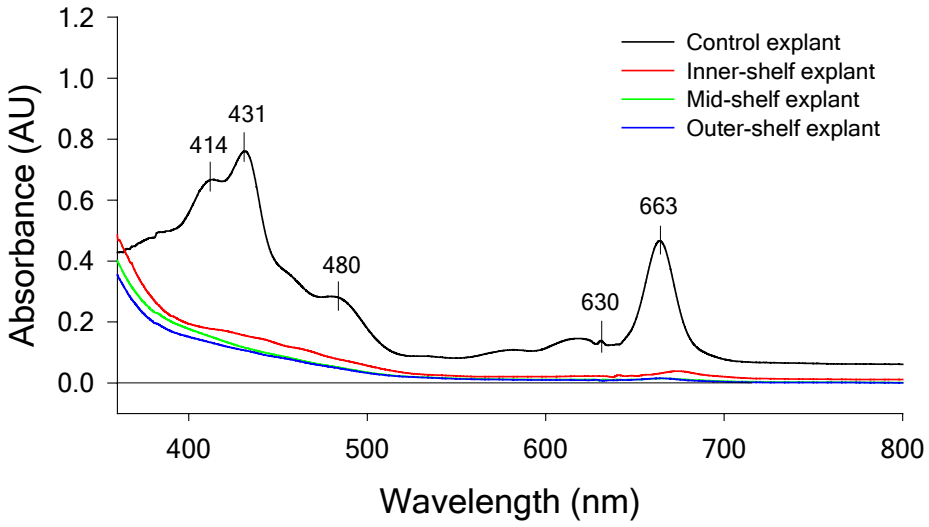

Figure 3.5 Mean absorbance spectra of acetone extracted pigments for inner- (n=10), mid- (n=10) and outer-shelf reef explants (n=10) of *R. odorabile* and for explants of *C. foliascens* (n=5). Chlorophyll a (664 nm), c_1 and c_2 (630 nm) (Jeffery and Humphrey 1975).

3.4 Discussion

The results from this study demonstrate that in contrast to previous implications *R. odorabile* across shelf locations of the central GBR do not photosynthesise. Although Webster and Hill (2001) identified the presence of cyanobacteria in the tissues of *R. odorabile*, the abundance of these cyanobacterial cells could not be empirically determined. This study shows that if cyanobacteria are present, they do not provide the sponge with any nutritional requirements through photosynthesis. The methods used by Webster and Hill (2001) to extract and isolate cyanobacteria from *R. odorabile* tissues collected directly from the field (within 15 min after collection) may have included cyanobacterial cells that were trapped in the filtering apparatus as a potential source of food. Cyanobacteria are a source of food for marine sponges in coral reef habitats (Pile 1997, 1999, 2005). Therefore, the identification of cyanobacterial symbionts from *R. odorabile* may have been an artefact of sampling, or alternatively they may be present in very low numbers in their tissues. The absence of photosymbionts within the sponge (as a holobiont) is further supported by the absence of photopigments in surface tissues of *R. odorabile* collected from reefs across inner-, mid- and outer-shelf locations.

Whilst the presence of cyanobacteria in sponge tissues has been linked to photosynthetic activity of many marine sponges (Wilkinson 1983; Cheshire and Wilkinson 1991; Cheshire et al. 1995, 1997) the mere presence of cyanobacteria in sponge tissues does not infer a photosynthetic response in sponges (Wilkinson 1983). Sponge-bacterial symbioses in marine sponges are complex with only a handful of studies elucidating the key roles of bacterial consortia to sponge physiology (Taylor et al. 2007b; reviewed in Taylor et al. 2007a). Whilst complex, several studies have demonstrated the importance of symbiotic relationships of sponges and microbial communities to UV protection (Wilkinson 1980; Regoli et al. 2000), enhanced boring and growth rates (Hill 1996), metabolite production (Hay 1996; Amsler et al. 2001; Paul and Puglisi 2004), and most importantly sponge

nutrition (Wilkinson 1983). However, in a broader sense, the role of most sponge-microbial associations remains unclear (reviewed in Taylor et al. 2007a).

As *R. odorabile* do not acquire additional energy through photosynthesis, energy and nutritional requirements are obtained through filter feeding. Therefore, it remains unclear what role light plays in determining the skewed depth distribution of *R. odorabile*, where abundance is highest within the phototrophic zone (8 - 12 m, Chapter 2) other than affecting larval settlement as described by Whalan et al. (in press). This finding is in contrast to other phototrophic and mixotrophic sponges found at similar depths on mid- and outer-shelf reefs of the central GBR which have a limited distribution that is restricted by a maximum depth coinciding with their photosynthetic compensation depth (Cheshire and Wilkinson 1991).

In conclusion, *R. odorabile* do not possess photo-symbionts, or they may not be present in sufficient numbers for light availability to aid or impede energy transfer processes for *R. odorabile*. However, a reduction in light availability on inner-shelf reefs may drive shallow depth distribution patterns of individuals due to *R. odorabile* larvae displaying positive photo-tactic behaviour prior to settlement, supporting settlement on exposed surfaces with high levels of light (Whalan et al. in press). Furthermore, as discussed in Chapter 2, other biotic and abiotic factors may contribute to variations in the distribution and abundance of *R. odorabile* across shelf locations of the central GBR. Persistent environmental gradients exist across the GBR lagoon, in particular a gradient of suspended sediments (Devlin and Brodie 2005). Understanding the impacts of these sediments (including grain sizes and mineralogical composition) on the distribution and abundance patterns of *R. odorabile* require rigorous examination. Therefore, a significant part of the remainder of this thesis focuses on evaluating how suspended sediments affect *R. odorabile*. In the subsequent chapter (Chapter 4), the size and mineralogy of suspended sediments across shelf locations of the central GBR are measured. This allows for comparisons between the abundance and distribution of

R. odorabile and suspended sediment properties within sponge habitats across shelf locations of the central GBR.

Chapter 4 – The Characterisation of Suspended Sediments within the Habitat of *Rhopaloeides odorabile*: A Cross Shelf Comparison

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4.1 Introduction

Water quality in inshore environments of the GBR has declined over the last century due to increased land clearing efforts from agricultural developments adjacent to the Queensland coast (Neil et al. 2002; McCulloch et al. 2003). This decline is attributed to a four-fold increase in nutrients (Neil et al. 2002) and up to a ten-fold increase in sediments entering inshore waters (McCulloch et al. 2003). Furthermore, fertilisers, heavy metals, herbicides and pesticides entering inshore waters via river discharges are also increasing (Haynes et al. 2000, 2005; Devlin and Brodie 2005; McMahon et al. 2005). The movement of suspended sediments, nutrients and pollutants from inner-shelf reefs to mid- and outer-shelf reefs on the central GBR are restricted (Brinkman et al. 2001; Luick et al. 2007). This restriction is a result of a combination of south east trade winds, east-

south east swell waves and the north-westward longshore movement of inner-shelf waters (Orpin et al. 1999; Brinkman et al. 2001; Larcombe and Carter 2004; Luick et al. 2007). This provides a distinct environmental gradient across the GBR shelf area. Hence, inner-shelf reefs are exposed to terrestrial sediments associated with terrigenous runoff, while mid- and outer-shelf reefs are dominated by carbonate sediments (Devlin and Brodie 2005).

Declining water quality in benthic habitats strongly impacts sessile marine organisms (Rogers 1990; Fabricius and Wolanski 2000) and can lead to changes in community structure, biomass and metabolism of benthic organisms (Balata et al. 2005). In inshore coral reef environments, increased sediment flux has resulted in the degradation of coral reef communities as evidenced by declining coral cover, reduced biodiversity, lower recruitment and a change in community structure (reviewed in Fabricius 2005). In addition, temperate rocky shore environments also show similar trends to coral reefs with reductions in species richness, diversity and abundance of benthic organisms with increased sediment flux (Airoldi and Cinelli 1997; Connell 2005).

The effects of sediments on coral physiology on coral reefs of the GBR have been well studied (reviewed in Fabricius 2005). Prolonged smothering of corals by fine sediment deposition kills exposed coral tissues, reduces photosynthetic yields, and in some cases increases metabolic costs associated with removing settled particles (reviewed in Fabricius 2005). Despite these negative impacts there is contradictory evidence suggesting that coral reef communities are adapting to these changing inshore environments, since they have been exposed to muddy conditions for the last few thousands of years (Smithers and Larcombe 2003). In addition, Anthony (2006) identified that corals inhabiting coastal high turbidity reefs have enhanced somatic energy reserves compared with coral inhabiting offshore reefs. This suggests that some corals may adapt to these unfavourable inshore conditions by storing excess lipids in their tissues, which may be used to maintain growth and survival when required.

In contrast to the increasingly rigorous examination of sediments and other human induced change on corals (reviewed in Lough 2008), the effects of sediments on sponge physiology and ecology on coral reefs are poorly understood. Increased levels of sedimentation have been reported to reduce growth rates (Lohrer et al. 2006; Roberts et al. 2006), impede reproduction (Roberts et al. 2006; Whalan et al. 2007a) and reduce clearance rates of sponges (Lohrer et al. 2006). In extreme cases, high concentrations of sediments induce prolonged reductions in pumping activity of sponges (Gerrodette and Flechsig 1979). In addition, sediment deposition also affects sponge morphologies (Maldonado and Uriz 1999; Cerrano et al. 2002; McDonald et al. 2002; Bell 2004) and influences the abundance, diversity and richness of sponge assemblages (Zea 1994; Bell and Barnes 2000a,b; Carballo 2006). However, it remains unclear whether the impact of sediments on sponge biology and ecology is related to the grain size or mineralogical composition of suspended sediments, or whether it is primarily a function of sediment deposition rates.

This chapter quantifies spatial and temporal variation in sediment deposition rates, and the size structure and mineralogical composition of inorganic suspended sediments associated with sponge habitats across the continental shelf of the central GBR. A particular focus will be placed on examining the potential role of suspended sediments in structuring the distribution and abundance of *R. odorabile* across shelf locations on the central GBR. The questions specifically addressed are: (1) Does the deposition rate of inorganic suspended sediments change seasonally across inner-, mid- and outer-shelf reefs? (2) Does the particle size distribution, mean grain size and mean skewness of inorganic suspended sediments change with respect to shelf location and seasons? (3) Does the particle size structure of inorganic suspended sediments change seasonally across inner-, mid- and outer-shelf reefs? (4) Does the mineralogical content of inorganic suspended sediments change seasonally across inner-, mid- and outer-shelf reefs?

4.2 Materials and methods

4.2.1 Study sites

To examine changes in sediment flux within sponge habitats across the central GBR, an east-west cross shelf transect was established. Three reefs, an inner-shelf reef (Pelorus Island, 18°33.85'S, 146°29.93'E), a mid-shelf reef (Rib Reef, 18°29.97'S, 146°52.52'E) and an outer-shelf reef (Pith Reef, 18°11.48'S, 146°29.93'E) were selected (Figure 4.1). These reefs were chosen, as they span the entire environmental gradient present across the GBR shelf (Devlin and Brodie 2005), and they are common habitats for *R. odorabile* (Chapter 2). Sediment traps were deployed at two replicate sites (separated by 150 m) of similar habitat structure at each reef.

The study sites at each reef were located on the south east exposed side, proximal to the major south east trade winds and wave inputs. These locations were chosen because previous studies have identified high numbers of *R. odorabile* in high-energy environments (Chapter 2 and Wilkinson and Cheshire 1989; Wilkinson and Evans 1989; Bannister et al. 2007). Sites were also the same as those used to collect sponge feeding samples carried out in Chapter 5.

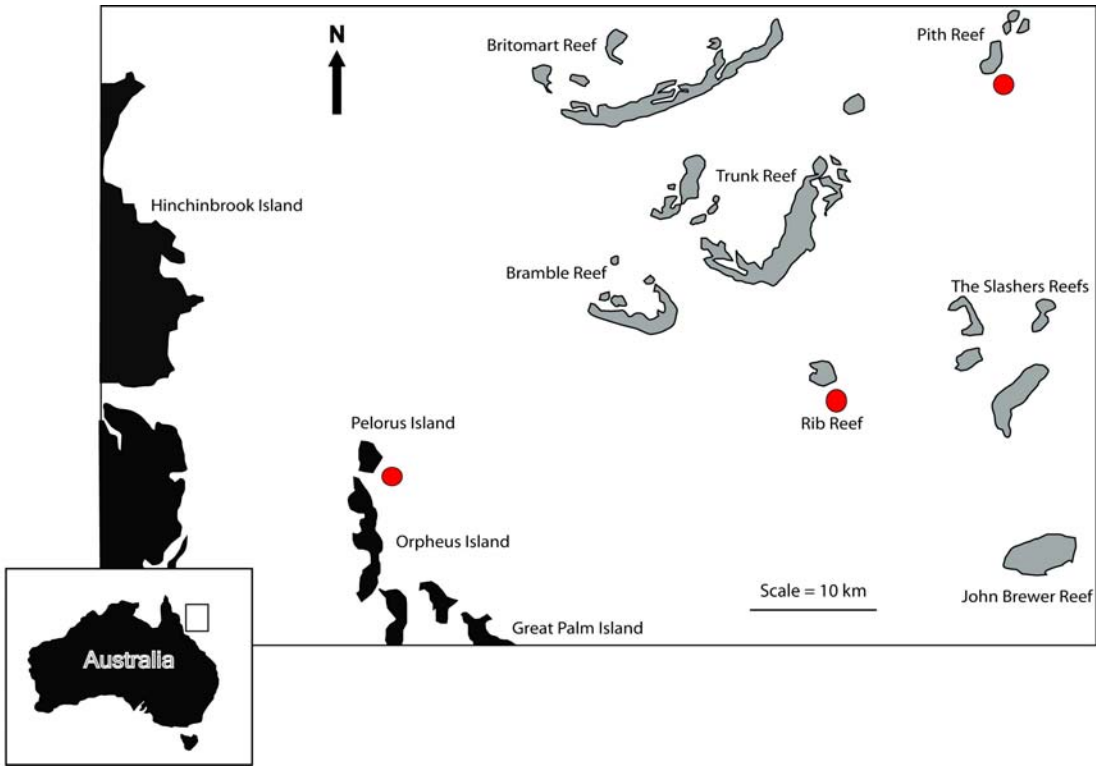

Figure 4.1 Map of reefs sampled for suspended sediments across inner-, mid- and outer-shelf locations of the central GBR, Australia. Red circles indicated reefs sampled. Black objects represent land and grey objects represent reef complexes.

4.2.2 Sediment grain size, abundance and mineralogy

4.2.2.1 Sediment traps

Suspended sediments were collected during the Austral winter (tropical dry season) 2005 and Austral summer (tropical wet season) 2005/06 in three replicate sediment traps at a depth of 10 m on the fore-reef slope at each coral reef location using a structured hierarchical sampling design (Figure 4.2). Three replicate sediment traps were mounted to individual star pickets separated by 1 m at each study site. Sediment traps remained at each study site for three successive 30 d intervals during winter (July, August and September) and summer (December, January and February). Sediment traps were capped underwater (to prevent sediment loss), removed and replaced with new sediment traps after each 30 d period.

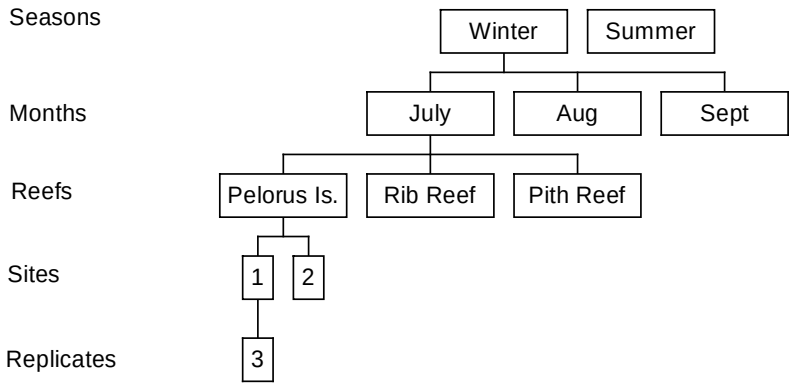

Figure 4.2 Hierarchical sampling design employed to determine cross shelf sedimentation characteristics on the central GBR, Australia.

Each sediment trap consisted of a cylindrical PVC container with a trapping area of 19.63 cm^2 mounted on supportive frames (Figure 4.3). Cylinder shaped traps with a height to width aspect ratio of 3:1 were chosen, because these are the most efficient traps for catching sediment and preventing re-suspension within traps (Butman 1986). Sediment traps were elevated 1 m above the benthos to minimise the effects of local turbulence generated from smaller structures, such as coral heads (<1 m height), into traps and the re-suspension of loose particles from the benthos into the traps (Figure 4.3). At each study site sediment traps were mounted at a depth of 10 m.

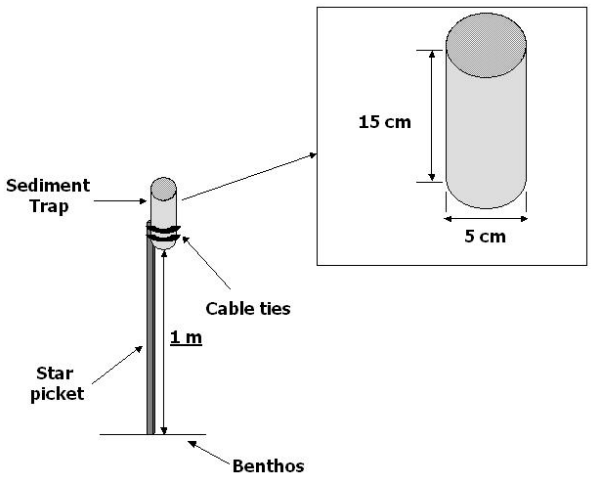

Figure 4.3 Diagram of sediment trap design with sediment trap mounted to star picket. Insert illustrates PVC sediment trap with external height and width measurements.

4.2.2.2 Sample storage and preparation

Sediment traps were collected and replaced at 30 d intervals. Sediment samples from each trap were placed into 100 ml specimen jars and stored in a cold room at 4°C until processed. Samples were analysed for grain size and mineralogical composition to quantify the composition and types of sediments present at each study site. Due to the length of time the sediments remained within the sediment traps (30 d), the organic fractions present in the samples may be at different stages of decomposition. Therefore, to obtain accurate measurements of inorganic sediment grain size and mineralogical composition, the organic fractions of each sample were removed with a treatment of 30% hydrogen peroxide (H_2O_2) prior to sediment characterisation (Jackson1985).

4.2.2.3 H_2O_2 treatment

All sediments collected in individual sediment traps were placed into individual 100 ml beakers and mixed with double distilled water. Beakers were placed into a water bath (60°C) for 24 h to accelerate the oxidisation reaction. Approximately 10 ml of 30% H_2O_2 solution was added to each sample. Samples were repeatedly stirred (using a magnetic stirrer) for 24 h. Once all organic material was dissolved, samples were removed from the water bath. Sediment samples were then allowed to settle at room temperature for 2 weeks before excess H_2O_2 supernatant was removed using a glass pipette.

4.2.2.4 Grain size analysis and total sediment weight

To determine the sediment grain size of each sediment sample collected (n=108), each sample was analysed on the Malvern Mastersizer 2000 laser particle sizer with a 0.02 - 2000 μm lens following manufacturers instructions (Malvern Instruments). Using this method grain size is directly proportional to the angle of incidence created when a laser beam hits sediment grains that are

moving through a transparent lens. The diffracting beams are re-directed onto a sizing detector board and the particle size is calculated. Before laser analysis, approximately 5 g of each sediment sample was washed through a 2000 μm sieve to ensure only sediment grains <2000 μm were analysed using the Mastersizer. However, none of the sediment samples collected had grain sizes >2000 μm. Once added the sample was kept representative in the chamber by automated continual stirring for the duration of the analysis, and the ultrasonic probe was activated for 30 s to de-coagulate the sample. The sample was then analysed. Once analysed the sample and liquid were drained from the Mastersizer and collected into 3 l beakers. The sample solution was then filtered onto pre-weighed 0.1 μm filter paper (Nucleopore) and placed into an oven at 60°C and dried to constant weight. The remainder of the sediment sample was placed into pre-weighed Petri dishes and placed into an oven at 60°C and dried to constant weight. Once dried all samples were weighed and data was recorded.

4.2.2.5 Mineralogical analysis

Mineralogical analysis was used to determine the mineralogical composition of the suspended sediments collected across the reef shelf during summer and winter, quantifying temporal and spatial changes in inorganic suspended sediment on the central GBR. To determine the mineralogical content of sediment from each study site a sub-sample (approximately 5 g) from one representative sediment sample from each site during each month of sampling (n = 36) was removed and crushed using a tungsten mill. These sediment samples were prepared as smear mounts where approximately 0.5 g of sample was mixed with distilled water and smeared onto a round glass slide (15 mm diameter). Each slide was inserted into separate cavity mounts and analysed on a Siemens D5000 Front-Loading X-ray Diffractometer (XRD). The XRD was fitted with a copper tube (Cu Kα = 1.54178 Å) operating at 30 kV and 20 mA and a post diffraction graphite monochromator. All samples were scanned from 1.3° to 65° in steps of 0.01° for 2.4 s per

step. The crystalline mineral phases of all samples analysed were quantified using SIROQUANT software (Taylor 1991).

4.2.3 Statistical analysis

All statistical analyses were performed using SYSTAT version 10. To ensure data met the assumptions of the statistical analyses performed, all data were checked for homogeneity of variance and normality using residuals versus predicted values plots and Q-Q plots of residuals, respectively. Data transformations for each statistical analysis are discussed below.

To determine significant differences in the deposition rate, mean grain size and mean skewness of suspended sediments, 3-factor partially nested ANOVA models were employed (Factors: (1) Month (fixed factor) – 6 months, (2) Shelf (fixed factor) – 3 shelf locations, (3) Sites nested within Month x Shelf (random factor) – 2 sites per shelf location. Deposition data was log (x + 1) transformed and mean grain size and mean skewness data were square root transformed to meet the assumptions of ANOVA (Underwood 1981). Significant differences were interpreted using Tukey's HSD multiple comparisons test (Quinn and Keough 2002). Furthermore, as months analysed were within specific seasons (i.e. months 1, 2, and 3 were winter and months 4, 5, and 6 were summer) planned contrast analyses were carried out to determine seasonal differences in the deposition rate, mean grain size and mean skewness of suspended sediments.

Similarly, 3-factor partially nested ANOVA models were employed to determine significant differences in the percent volume of suspended sediments within three distinct size classes (<10 μm, 10 – 100 μm, and >100 μm) (Factors: (1) Month (fixed factor) – 6 months, (2) Shelf (fixed factor) – 3 shelf locations, (3) Sites nested within Month x Shelf (random factor) – 2 sites per shelf location). Data for the <10 μm and >10 μm size classes were arcsin square root transformed to meet the assumptions of ANOVA (Underwood 1981). Significant differences were interpreted using

Tukey's HSD multiple comparisons test (Quinn and Keough 2002). Furthermore, planned contrast analyses were also carried out to determine seasonal differences in the percent volume of suspended sediments within the three distinct size classes. Due to multiple testing, a Bonferroni transformed experiment-wise $\alpha = 0.0166$ was employed to reduce type 1 error rates (Quinn and Keough 2002).

To examine differences in the percent weight of suspended sediments within three distinct mineralogical classes (carbonate, clay, and quartz) 3-factor partially nested ANOVA models were employed (Factors: (1) Season (fixed factor) – 2 seasons, (2) Shelf (fixed factor) – 3 shelf locations, (3) Month nested within Season x Shelf (fixed factor) – 3 months per season). Each mineralogical type was arcsin square root transformed to meet the assumptions of ANOVA (Underwood 1981). Significant differences were interpreted using Tukey's HSD multiple comparisons test (Quinn and Keough 2002). Due to multiple testing a Bonferroni transformed experiment-wise $\alpha = 0.0166$ was employed to reduce type 1 error rates (Quinn and Keough 2002).

4.3 Results

4.3.1 Sediment deposition

The deposition rates of suspended sediments varied significantly between sponge habitats as well as between months (Figure 4.4 and Table 4.1). The deposition rates within sponge habitats on outer-shelf reefs (ranging between 25.3 and 42.0 $g.m^{-2}.d^{-1}$) were on average 4 and 5 times higher than within sponge habitats on mid- (ranging between 4.6 and 12.6 $g.m^{-2}.d^{-1}$) and inner-shelf reefs during winter (ranging between 6.6 and 9.1 $g.m^{-2}.d^{-1}$), respectively (Figure 4.4). However, during summer deposition rates remained similar between sponge habitats on inner- (ranging between 3.7 and 6.8 $g.m^{-2}.d^{-1}$), mid- (ranging between 1.4 and 5.3 $g.m^{-2}.d^{-1}$) and outer-shelf reefs (ranging between 0.6 and 10.0 $g.m^{-2}.d^{-1}$) (Figure 4.4). High variation in the deposition rates on outer-shelf reefs between

the summer and winter months explains the significant interaction term (month x shelf) with considerably higher deposition rates during the winter months than the summer months (Table 4.1).

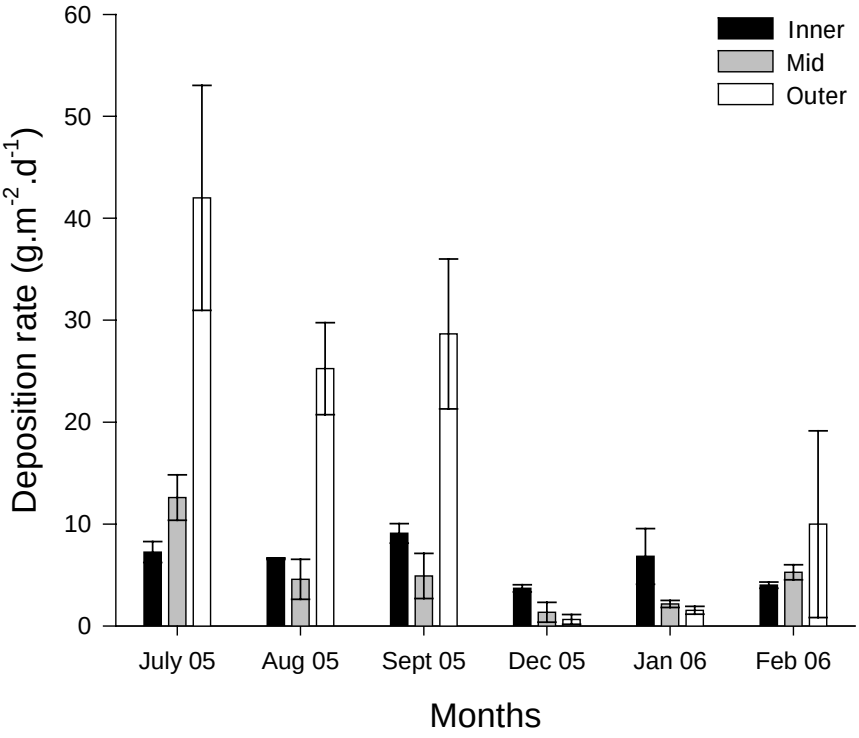

Figure 4.4 Mean sediment deposition rate ($g.m^{-2}.d^{-1}$ ± SE) during monthly sampling periods across inner-, mid- and outer-shelf locations of the central GBR.

Table 4.1 Three-factor partially nested ANOVA model for log (x + 1) transformed deposition rates of suspended sediments across inner-, mid- and outer-shelf locations of the central GBR, Australia. (Month: July, August, September, December, January and February; Shelf: Inner-, mid- and outer-shelf locations; Sites: 2 sites).

Source	df	MS	F	*P*
Month	5	9.277	14.12	< 0.001
Shelf	2	2.591	3.95	0.038
Month x Shelf	10	2.432	3.70	0.008
Sites (Month x Shelf)	18	0.657	-	No test
Error	72	0.561		

4.3.2 Sediment grain size distribution

The mean sediment grain size distributions differed between shelf locations within each season (Figure 4.5a,b) but show minimal variation within each shelf location, indicating suspended

sediment stability and uniformity over time. During winter, sediment grain size distributions on the inner- and mid-shelf reefs were distinctly bi-modal. These modes were dominated by the ~7 μm (2.7 vol-%) and ~250 μm fractions (2.2 vol-%) on inner-shelf locations, while on the mid-shelf location, these modes were dominated by ~56 μm (1.8 vol-%) and ~400 μm fractions (3.4 vol-%). On outer-shelf locations, sediment grain size distributions were uni-modal with a dominant ~400 μm fraction (5.9 vol-%) (Figure 4.5a).

During summer, sediment grain size distributions on the inner- and mid-shelf reefs were uni-modal. On inner-shelf reefs the dominant mode was the ~7 μm fraction (3.6 vol-%), whereas on the mid-shelf reef the dominant mode was the 500 μm fraction (2.9 vol-%). However, on the outer-shelf reef the sediment grain size distribution was bi-modal with these modes dominated by the ~280 μm (2.9 vol-%) and ~560 μm (3.0 vol-%) fractions (Figure 4.5b).

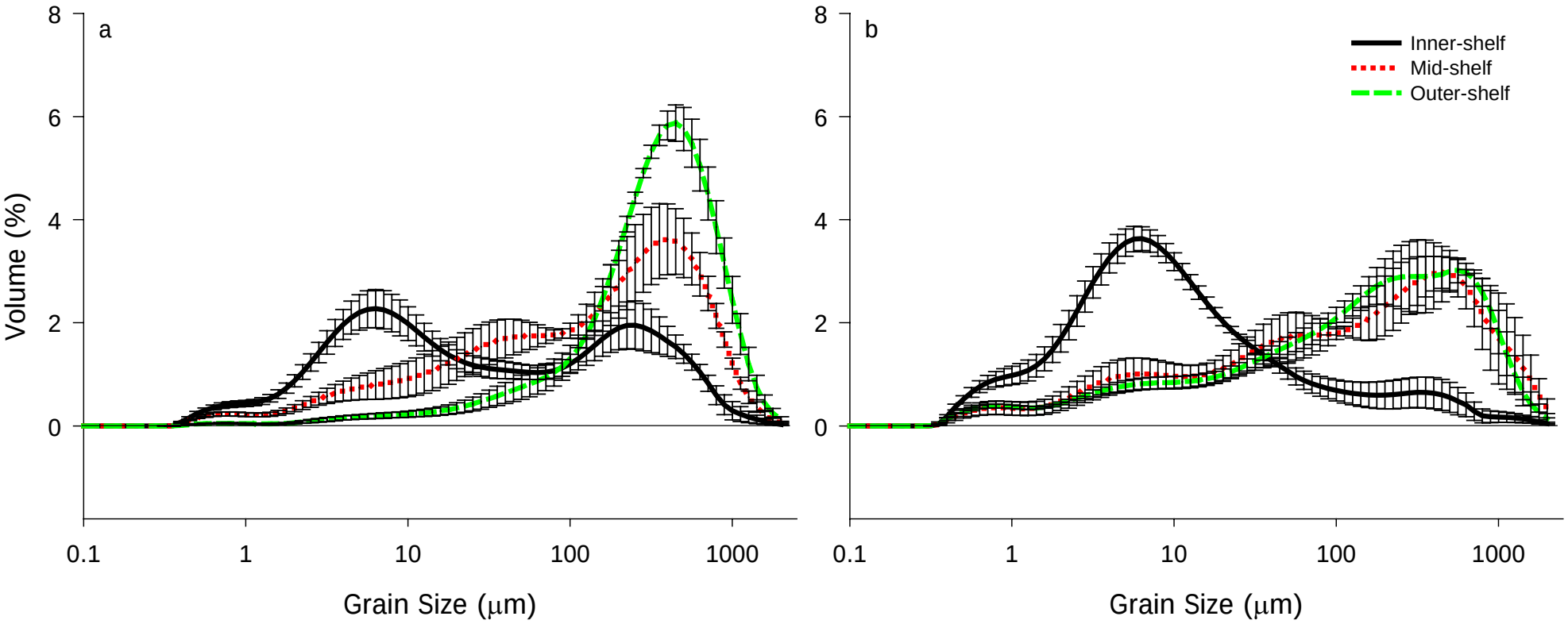

Figure 4.5 Mean grain size distribution (± SE) of suspended sediment collected in sediment traps on inner-, mid- and outer-shelf locations during (a) winter and (b) summer (n=3). Mean grain size distribution data for each season was calculated by averaging mean grain size distribution data for each month of sampling at each shelf location.

The mean grain size (Figure 4.6a) of suspended sediments differed significantly across inner-, mid- and outer-shelf reef locations on the central GBR (Table 4.2a). However, no seasonal differences were found (Planned contrast analysis; mean grain size, $F_{(1,72)} = 0.011$, $P = 0.917$). The mean grain sizes of suspended sediment on outer-shelf reefs were dominated by coarse sediment fractions (214.3 ± 33.4 μm) and were 1.7 times larger than mid-shelf reefs that were also dominated by coarse sediment fractions (126.6 ± 30.5 μm). Outer-shelf reefs had mean suspended sediment grain sizes 8 times larger than inner-shelf reefs that were dominated by fine-grained sediments (24.4 ± 5.7 μm). Furthermore, the mean skewness (Figure 4.6b) of suspended sediment distributions also differed spatially across the central GBR (Table 4.2b). However, no seasonal differences were found (Planned contrast analysis; Mean skewness, $F_{(1,72)} = 0.052$, $P = 0.820$). Grain size distributions on inner-shelf reefs were positively skewed (0.2 ± 0.1) compared with mid- (-0.9 ± 0.2) and outer-shelf reefs (-1.4 ± 0.2) that had negatively skewed grain size distributions.

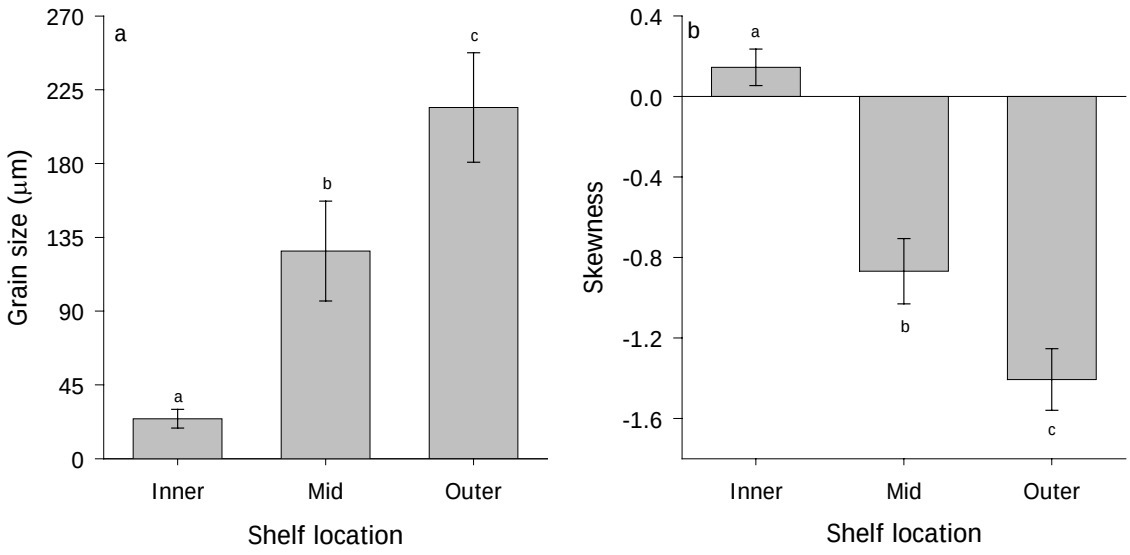

Figure 4.6 (a) Mean grain size (μm, ± SE) and (b) mean skewness (± SE) of suspended sediments collected in sediment traps at inner-, mid- and outer-shelf locations across the central GBR, Australia. Superscripts denote significant differences (Tukey's HSD multiple comparison test, $P < 0.05$ for square root transformed mean grain size and mean skewness data, n=6 for each shelf location).

Table 4.2 Three-factor partially nested ANOVA models for (a) square root transformed mean grain size and (b) square root transformed mean skewness of suspended sediment collected in sediment traps at inner-, mid- and outer-shelf locations on the central GBR, Australia. (Month: July, August, September, December, January and February; Shelf: Inner-, mid- and outer-shelf locations; Sites: 2 sites).

Source	df	MS	F	*P*
(a) Mean grain size				
Month	5	78.862	1.77	0.170
Shelf	2	629.104	14.11	0.000
Month x Shelf	10	60.715	1.36	0.273
Sites (Month x Shelf)	18	44.600	-	No test
Error	72	12.766		
(b) Mean skewness				
Month	5	0.188	2.34	0.078
Shelf	2	2.225	28.44	0.000
Month x Shelf	10	0.114	1.46	0.235
Sites (Month x Shelf)	18	0.078	-	No test
Error	72	0.038		

The percentage of sediments in each size class differed significantly across shelf location (Figure 4.7a,b and Table 4.3)**.** On inner-shelf locations up to 80% of the total suspended sediments were made up of <100 μm size fractions in comparison to mid- and outer-shelf locations where the <100 μm fraction made up only 45% and 30%, respectively, of the total suspended sediments. Although suspended sediments <10 μm exhibited some monthly variation (Table 4.3) there was no seasonal difference in the fraction of suspended sediments within each of the three size classes (Planned contrast analysis; <10 μm, $F_{(1,72)} = 1.128$, $P = 0.292$; 10 - 100 μm, $F_{(1,72)} = 3.186$, $P = 0.078$; and >100 μm, $F_{(1,72)} = 0.108$, $P = 0.744$).

The amount of suspended sediments with grain sizes <10 μm were significantly greater on inner-shelf locations (48.6 ± 3.7%) compared to mid- (15.4 ± 2.5%) and outer-shelf locations (8.5 ± 2.2%) (Figure 4.7a). The amount of suspended sediment with grain sizes between 10 - 100 μm were similar between inner- (31.1 ± 1.4%) and mid-shelf locations (29.6 ± 3.7%), but significantly different between inner- and outer-shelf locations (19.5 ± 3.5%) (Figure 4.7b). Furthermore, the suspended sediments >100 μm differed significantly between inner- (20.3 ±

4.2%), mid- (55.0 ± 5.9%) and outer-shelf locations (72.0 ± 5.3%), with mid- and outer-shelf locations having significantly more particles >100 μm than inner-shelf reefs (Figure 4.7c).

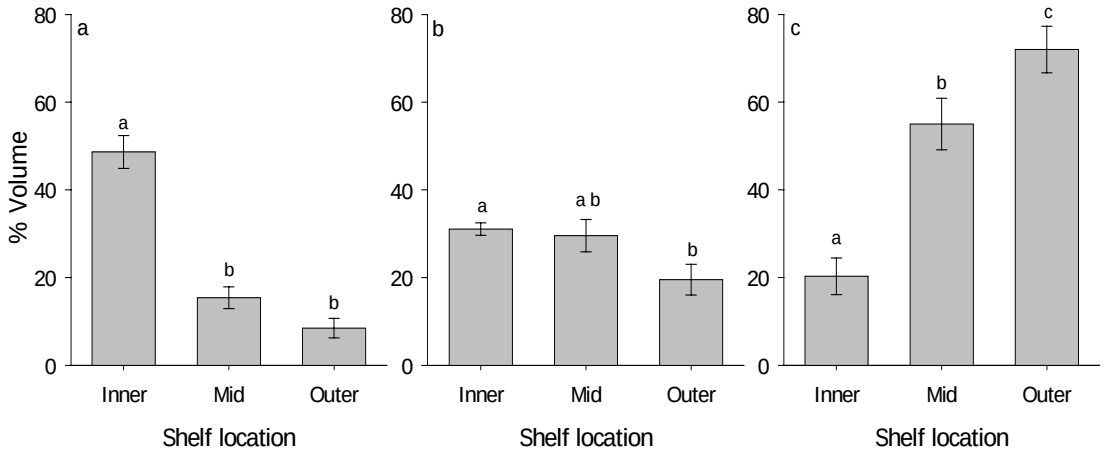

Figure 4.7 Mean % volume (± SE) of suspended sediment (a) <10 μm, (b) 10 – 100 μm and (c) >100 μm collected in sediment traps across inner-, mid- and outer-shelf locations on the central GBR, Australia. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05 for arcsin square root transformed data of <10 and >100 μm size fractions, n=6 for each shelf location).

Table 4.3 Three-factor partially nested ANOVA models for mean percent volume of (a) arcsine square root transformed suspended sediment <10 μm, (b) suspended sediment 10 - 100 μm and (c) arcsine square root transformed suspended sediment >100 μm collected at inner-, mid- and outer-shelf locations on the central GBR, Australia. (Month: July, August, September, December, January and February; Shelf: Inner-, mid- and outer-shelf locations; Sites: 2 sites). Bonferroni corrected $\alpha = 0.05/3 = 0.0166$.

Source	Df	MS	*F*	*P*
(a) <10 μm				
Month	5	0.167	4.93	0.005
Shelf	2	2.561	75.66	0.000
Month x Shelf	10	0.034	1.01	0.475
Sites (Month x Shelf)	18	0.034	-	No test
Error	72	0.024		
(b) 10-100 μm				
Month	5	432.049	1.60	0.210
Shelf	2	1451.937	5.38	0.015
Month x Shelf	10	235.684	0.87	0.573
Sites (Month x Shelf)	18	269.834	-	No test
Error	72	134.632		
(c) >100 μm				
Month	5	0.444	2.30	0.089
Shelf	2	2.622	13.55	0.000
Month x Shelf	10	0.259	1.34	0.284
Sites (Month x Shelf)	18	0.194	-	No test
Error	72	0.051		

4.3.3 Mineralogical composition

The mineralogical composition of the suspended sediments showed little seasonal variation, although large variations were found across shelf locations of the central GBR (Figure 4.8a,b,c and Table 4.4). At all sites sampled the trapped sediments were dominated by carbonates with inner-shelf sites having significantly lower percentages (53.17 ± 3.17%) than mid- (95.41 ± 0.42%) and outer-shelf sites (99.67 ± 0.34%) (Figure 4.8a). Furthermore, suspended siliciclastic sediments were also abundant on inner-shelf environments with high amounts of clay (31.57 ± 0.59%) and quartz mineral (10.42 ± 0.85%). In contrast, mid- and outer-shelf environments had only trace levels of clay (2.84 ± 0.34% and 0.08 ± 0.08%, respectively) and quartz mineral (1.75 ± 0.08% and 0.25 ± 0.25%, respectively) (Figure 4.8b,c).

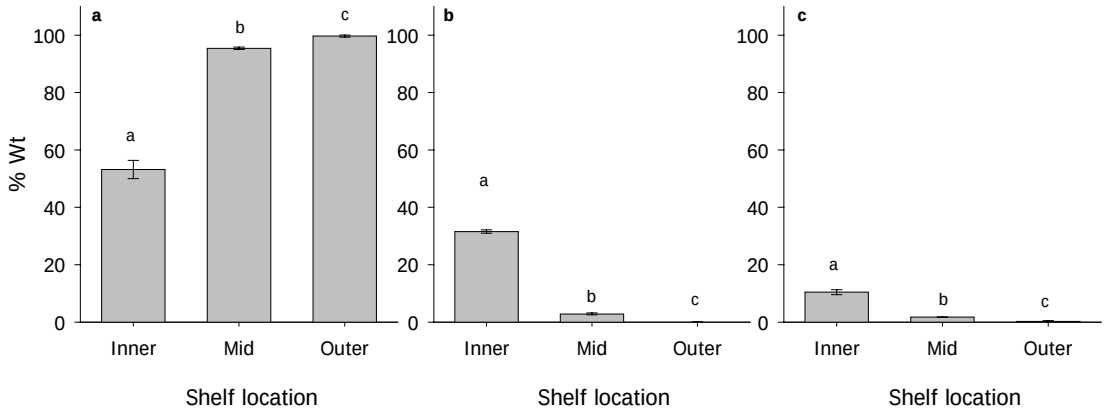

Figure 4.8 Mean percent weight (%-wt, ± SE) of (a) carbonate, (b) clay and (c) quartz composition of suspended sediment collected in sediment traps across inner-, mid- and outer-shelf locations on the central GBR, Australia. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05 for arcsin square root transformed data, n=6 for each shelf location).

Table 4.4 Three-factor partially nested ANOVA models for the mean percent weight of arcsin square root transformed (a) carbonate, (b) clay and (c) quartz composition of suspended sediments collected at inner-, mid- and outer-shelf locations on the central GBR, Australia. (Month: July, August, September, December, January and February; Shelf: Inner-, mid- and outer-shelf locations; Sites: 2 sites). Bonferroni corrected $\alpha = 0.05/3 = 0.0166$.

Source	df	MS	F	*P*
(a) Carbonate				
Season	1	0.182	1.25	0.287
Shelf	2	1.111	7.60	0.007
Season x Shelf	2	0.021	0.14	0.868
Month (Season x Shelf)	12	0.146	-	No test
Error	30	0.129		
(b) Clay				
Season	1	0.009	0.17	0.689
Shelf	2	1.333	112.29	<0.001
Season x Shelf	2	0.017	1.41	0.282
Month (Season x Shelf)	12	0.012	-	No test
Error	30	0.010		
(c) Quartz				
Season	1	0012	3.71	0.078
Shelf	2	0.283	86.56	<0.001
Season x Shelf	2	0.000	0.13	0.879
Month (Season x Shelf)	12	0.003	-	No test
Error	30	0.004		

Despite significantly higher percentages of carbonate sediments on mid- and outer-shelf reefs than inner-shelf reefs the compositions of carbonate sediments across shelf locations were

similar, being comprised of aragonite, calcite and high magnesium calcite (Figure 4.9). These carbonate sediments differed proportionally across shelf locations with aragonite and high magnesium calcite occurring in higher proportions on mid- (49.9 ± 3.4 and 36.9 ± 0.8%-wt, respectively) and outer-shelf locations (60.6 ± 4.6 and 30.7 ± 4.4%-wt, respectively) compared to inner-shelf locations (26.4 ± 0.2 and 20.8 ± 3.3%-wt, respectively). Calcite occurred in similar proportions across inner- (6.1 ± 0.3%-wt), mid- (8.7 ± 2.2%-wt) and outer-shelf locations (8.4 ± 0.1%-wt) (Figure 4.9).

Although inner-shelf reefs have significantly greater percentages of clay minerals than mid-shelf reefs, the compositions of clays on inner- and mid-shelf locations are similar being comprised of kaolinite, illite/muscovite, and expanding clays (Figure 4.9). However, they differed proportionally with inner-shelf locations having a greater proportion of kaolinite, illite/muscovite and expanding clays (14.7 ± 0.9, 12.9 ± 2.1, and 3.9 ± 0.6%-wt, respectively) than mid-shelf locations (0.8 ± 0.2, 1.6 ± 0.3 and 0.4 ± 0.1%-wt, respectively) (Figure 4.9). In addition, suspended sediments in inner-shelf locations were also comprised of other terrestrial particles, including sodium plagioclase (4.4 ± 3.1%-wt) and trace levels of gypsum (0.4 ± 0.4%-wt), which were absent in suspended sediments across mid- and outer-shelf locations.

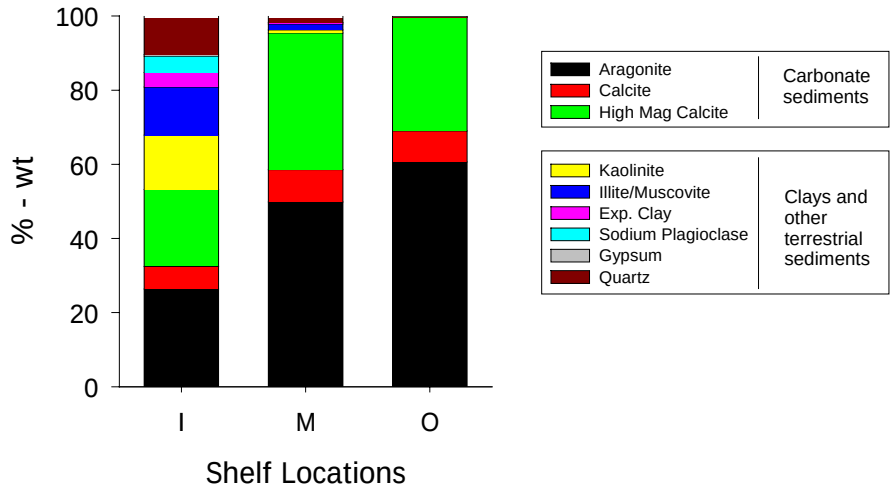

Figure 4.9 The percent weight (%-wt) of each type of carbonate, clay and terrestrial particles present in the composition of suspended sediments collected in sediment traps across inner-, mid- and outer-shelf locations of the central GBR, Australia.

4.4 Discussion

This study characterised and measured, for the first time, the deposition rate, size structure and mineralogical composition of inorganic sediments associated with sponge habitats across a tropical mixed-siliciclastic-carbonate platform (the GBR). Although deposition rates of suspended sediments varied between shelf locations and within and between seasons, there was a clear gradient in terms of both grain size and mineralogical composition of suspended inorganic sediments across the GBR shelf locations. Within inner-shelf locations suspended sediments consisted of up to 80% fine-grained sediments with grain sizes mostly <100 μm, which comprised of both terrestrial (clay and quartz) and biogenic material (carbonates). In contrast, mid-shelf locations contained only a small proportion of terrestrial material (up to 5%) with sediments consisting primarily of carbonates. Outer-shelf locations were exclusively carbonates. Up to 70% of suspended sediments across mid- and outer-shelf reefs had grain sizes of >100 μm. This study clearly provides correlative evidence linking sediment grain size and mineralogy to changes in the abundance and distribution of *R. odorabile* across shelf locations of the central GBR.

The size and mineralogical composition of suspended sediments within sponge habitats across reef shelf locations of the central GBR identified during this study are similar to previous studies that have characterised surface sediments adjacent to reef platforms on the central GBR (Woolfe et al. 2000; Orpin et al. 2004). These studies identified that fine-grained terrestrial sediments dominate coastal habitats adjacent to major river systems, while coarse carbonate sediments dominate surface sediments adjacent to mid- and outer-shelf reef habitats (Orpin et al. 2004). Although both studies comprehensively characterise shelf sediments on the central GBR, temporal variation was not investigated. However, this study clearly demonstrated that within shelf locations grain size and mineralogical composition of suspended sediments remain stable regardless of season.

Changes in sponge distribution, abundance and species richness (Zea 1994; Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006), morphology (Palumbi 1984; Bell et al. 2002; Cerrano et al. 2002; McDonald et al. 2002; Bell 2004), growth rates (Roberts et al. 2006), reproduction (Roberts et al. 2006; Whalan et al. 2007a), physiology (Gerrodette and Flechsig 1979; Maldonado and Uriz 1999) and survival of sponges (Maldonado et al. 2008) have been attributed to sedimentation. Despite increased rates of sedimentation being proposed as one of the key factors influencing the ecology of sponge assemblages (Zea 1994; Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006) there is a dearth of information examining the effects of mineralogical composition and sediment grain sizes on sponge physiology and ecology. This is in contrast to the numerous studies addressing the impacts of suspended sediments and their properties on coral physiology and ecology (reviewed in Fabricius 2005; Weber et al. 2006).

Distinct shifts in grain size and mineralogical composition of suspended sediments across inner-, mid- and outer-shelf locations may explain contrasting patterns of abundance, size and distribution of *R. odorabile* across depths and shelf locations of the central GBR (Chapter 2). The dominance of suspended fine clay and terrestrial sediments across inner-shelf reefs habitats and their continual re-suspension due to wave activity and currents associated with persistent south east trade winds (Larcombe et al. 1995) may restrict the recruitment of *R. odorabile* larvae to certain depths within inner-shelf reef locations compared to mid- and outer-shelf reef locations. For example, Whalan et al. (in press) identified that larvae from *R. odorabile* preferentially settled in light exposed habitats, irrespective of substrate. However, on inner-shelf reefs the re-suspension of fine clay sediments and other particles present in the water (turbidity) accounts for about 95% of the variability in irradiance levels with increasing depth (Anthony et al. 2004). As a result, the availability of light on inner-shelf reefs is reduced considerably compared with similar depths on mid- and outer-shelf reefs. Therefore, reduced depth distribution of *R. odorabile* on inner-shelf reef locations may be a function of increased

fine clay suspended sediments reducing the availability of light and consequently the region of reef for preferential settlement and potentially post-settlement survival.

The increased settlement of fine clay and terrestrial sediments on inner-shelf reefs may also reduce the abundance of *R. odorabile*. Firstly, it can do so by reducing the available space for sponge larvae to settle (Bell and Smith 2004). Second, sediments can smother and bury newly settled sponge recruits as well as established individuals (Ilan and Abelson 1995; Wulff 1997). In addition, reduced abundances of *R. odorabile* on inner-shelf reefs may be explained by the significantly lower reproductive output compared to mid- and outer-shelf reefs (Whalan et al. 2007a). Increased siltation and exposure to fine sediments have previously been shown to reduce the reproductive output of the temperate sponge *Cymbastela concentrica* (Roberts et al. 2006). Although there is no experimental evidence detailing the impacts of fine suspended clay and terrestrial sediments on the reproductive output of tropical sponges, Whalan et al. (2007a) provided correlative evidence linking increased turbidity on inner-shelf coral reefs to reduced reproductive output of *R. odorabile*. Both Roberts et al. (2006) and Whalan et al. (2007a) proposed that the reproductive output of sponges is compromised as a result of increased energy drains due to higher maintenance costs associated with living in sub-optimal habitats exposed to excessive siltation and turbidity. Therefore, persistent exposure of *R. odorabile* to increased suspended fine clay and terrestrial sediments on inner-shelf reefs may explain their reduced reproductive output, and subsequently their reduced abundance on inner-shelf reefs compared to mid- and outer-shelf reefs. However, the energetic cost associated with living in these sub-optimal habitats requires further experimentation to elucidate the causal relationship between the increased suspension of fine clay and terrestrial sediments and increased energy expenditure.

On inner-shelf reefs, exposure of *R. odorabile* to excessive fine clay suspended sediments (sediments with grain sizes of <100 μm) may clog their inhalant canals and filtering structures (Gerrodette and Flechsig 1979), and accumulate on their surfaces resulting in burial (Wulff

1997). In response to these conditions *R. odorabile* may initiate, like other sponges, active and passive responses to deal with these fine clay sediments (Bell 2004). Active responses to elevated levels of suspended sediments include mucus production (Turon et al. 1999), water flow reversal (Simpson 1984) and reductions in pumping activity (Gerrodette and Flechsig 1979). Passive responses include gross morphological modifications (Bell et al. 2002; Bell 2004), oscula re-positioning (Bell 2004) and structural modifications (Barthel and Tendal 1993; McDonald et al. 2002). Initiating these responses may be metabolically draining for *R. odorabile* and once again there have been no studies detailing the metabolic costs associated with these active and passive responses for sponges.

One response mechanism to high levels of sediment is the production of mucus to trap sediment. Mucus production in corals is metabolically very draining and can cost corals up to 2.5 times the amount of carbon they produce in 24 h (Riegl and Branch 1995). Mucus production by sponges may also be energetically demanding, however, despite visual confirmation of mucus production by sponges in response to sedimentation (Turon et al. 1999) it remains unclear what energetic costs are associated with the production of mucus by sponges. It is also unclear whether the energetic cost of mucus production changes significantly when exposed to suspended sediments that differ both with size and mineralogical composition. Therefore, to elucidate the differential energetic constraints of sponges exposed to contrasting grain sizes and mineralogical composition of suspended sediments, further manipulative experimentation is needed. In addition to mucus production, exposure to increased suspended fine sediment induces reductions in pumping activity of tropical sponges (Gerrodette and Flechsig 1979). Although this mechanism may be initiated to prevent clogging of inhalant canals and filtering structures to conserve energy (Gerrodette and Flechsig 1979) it has been suggested that reduced pumping activity may also reduce feeding efficiencies of sponges, and in turn reduce their consumption of energy (Reiswig 1971b; Gerrodette and Flechsig 1979). Despite these earlier suggestions there have been few studies characterising the impact of sediments, in particular grain size and the mineralogical composition of suspended sediments

on the retention efficiencies of ultraplankton by sponges. However, one study has provided initial evidence of reduced clearance rates of phytoplankton by sponges in response to elevated sedimentation (Lohrer et al. 2006).

In conclusion, there are significant differences in the deposition rate, size structure and mineralogical composition of suspended sediments across inner-, mid- and outer-shelf reef locations of the central GBR. These differences provide evidence that both grain size and mineralogy of suspended sediments may affect the ecology of sponges at an individual scale. However, it is difficult to determine a direct or indirect relationship between differences in grain size and mineralogy of suspended sediments to changes in the abundance and depth distributions of *R. odorabile* across shelf locations of the central GBR without appropriate experimental tests. The presence of fine suspended clay sediments on inner-shelf reefs (i.e. increased turbidity) is highly likely to be a contributing factor to the reduced abundance and depth distribution of *R. odorabile* on inner-shelf reefs. That the abundance and depth distribution of *R. odorabile* increases significantly with increasing distance from terrestrial influences further reinforces this proposal. Therefore, the findings from this study highlight the importance of considering both the grain size and mineralogy of suspended sediments when discussing or testing the effects of sedimentation on the biology and ecology of sponges.

Chapter 5 – Spatial and Temporal Variation in Feeding by *Rhopaloeides odorabile* Across a Natural Environmental Gradient

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5.1 Introduction

This chapter builds on the findings of Chapter 2, which characterised the abundance and distribution patterns of *R. odorabile* across reef shelf locations of the central GBR. As discussed in detail in Chapters 2 and 4, increased sedimentation, nutrients and pollutants and their limited movement from inshore to offshore reef habitats have resulted in the establishment of environmental gradients across the continental shelf of the central GBR (Devlin and Brodie 2005; Haynes et al. 2005; Lucik et al. 2007). Increased nutrient loads in inshore habitats (McKergow et al. 2005) have resulted in a significant increase in chlorophyll *a* concentrations (Brodie et al. 1997; Cooper et al. 2007) and may be responsible for cross-shelf phytoplankton composition and community dynamics (Revelante et al. 1982; Furnas and Mitchell 1986); key factors linking the structure and dynamics of sessile

benthic filter feeding invertebrates through benthic-pelagic coupling (Pile et al. 1997; Gili and Coma 1998; Coma and Ribes 2003; Lesser 2006). Again, despite detailed investigations of the negative impacts of environmental gradients on corals (reviewed in Fabricius 2005) few studies have investigated the impact of environmental gradients on sponges.

Sponges are highly efficient filter feeders, obtaining their nutritional requirements by feeding on a broad range of prey items (Reiswig 1981; Yahel et al 2006). Sponges predominately retain ultraplankton (the fraction of plankton <5 μm, consisting of prokaryotes and eukaryotes) with high efficiencies across many different benthic habitats, from temperate (Pile et al. 1996; Bell et al. 1999; Ribes et al. 1999a) and tropical shallow waters (Reiswig 1971a; Pile 1997, 1999, 2005) to deep-sea habitats (Pile and Young 2006; Yahel et al. 2006, 2007). In addition, sponges filter and retain larger phytoplankton species (Ribes et al. 1999a; Thurber 2007) and marine viruses (Hadas et al. 2006). Intrinsic associations with microbial symbionts have also allowed sponges to exploit other nutritional pathways including the assimilation of dissolved organic carbon and nitrogen, the retention of photosynthetically derived carbon and the consumption of methanotrophs (reviewed in Taylor et al. 2007a). Despite many studies identifying a suite of nutritional pathways employed by sponges to meet their metabolic requirements, *in situ* work examining temporal and spatial variation in feeding has received considerably less attention. Of the few studies examining temporal and spatial feeding patterns of sponges, food availability has been suggested as the main factor responsible for variation in the retention efficiency of prey items (Ribes et al. 1999a; Bell et al. 1999; Coma et al. 2001). However, the role of environmental gradients on food availability and the subsequent effects on sponge feeding and energy acquisition have largely been ignored.

Given the abundance and broad distribution of *R. odorabile* across shelf locations exposed to a defined environmental gradient (see Chapters 2 & 4), this chapter uses *R. odorabile* as a model species to investigate the effects of natural environmental gradients on the availability, selection and

retention efficiencies of ultraplankton <3 μm commonly consumed by shallow water marine demosponges. It is proposed that *R.odorabile* like other shallow water demosponges will feed unselectively on a range of ultraplankton cell types. Specifically this chapter addresses (1) What ultraplankton cell types are available to, and consumed by, *R. odorabile*? (2) Does the retention efficiencies of these ultraplankton cell types by *R. odorabile* change both spatially and temporally in relation to the environmental gradient across shelf locations? (3) Does the retention of carbon and nitrogen differ across shelf locations sampled?

5.2 Materials and methods

5.2.1 Study sites

Two east west cross-shelf transects were established to examine spatial variation in the retention efficiencies of ultraplankton by *R. odorabile* on the central GBR. Both transects included an inner-shelf (within 20 km from the coastline), a mid-shelf (40 to 60 km from the coastline) and an outer-shelf reef location (>60 km from the coastline). Cross-shelf locations are the same as those classified by Wilkinson and Cheshire (1989). They were chosen as they span the environmental gradient present across the reef shelf of the central GBR (Devlin and Brodie 2005) and are common habitats for *R. odorabile* (Chapter 2). At each shelf location, two reefs were independently chosen. Outer-shelf reefs included Pith (18°11.48'S, 146°29.93'E) and Needle Reefs (18°21.30'S, 147°12.21'E); mid-shelf reefs included Roxbrough (18°25.53'S, 147°03.74'E) and Rib Reefs (18°29.97'S, 146°52.52'E); and the inner-shelf reefs included fringing reefs surrounding Pelorus (18°33.85'S, 146°29.93'E) and Curacoa Islands (18°40.27'S, 146°34.06'E) (Figure 5.1). Sites sampled at Pith Reef, Rib Reef and Pelorus Island were also the same sites used in Chapter 4 for suspended sediment characterisation within sponge habitats. All sampling took place at the exposed locations (south east face, proximal to the major south east trade winds and wave inputs) at each

reef. *R. odorabile* occurs in high numbers in these high-energy environments (See Chapter 2; Wilkinson and Evans 1989; Bannister et al. 2007).

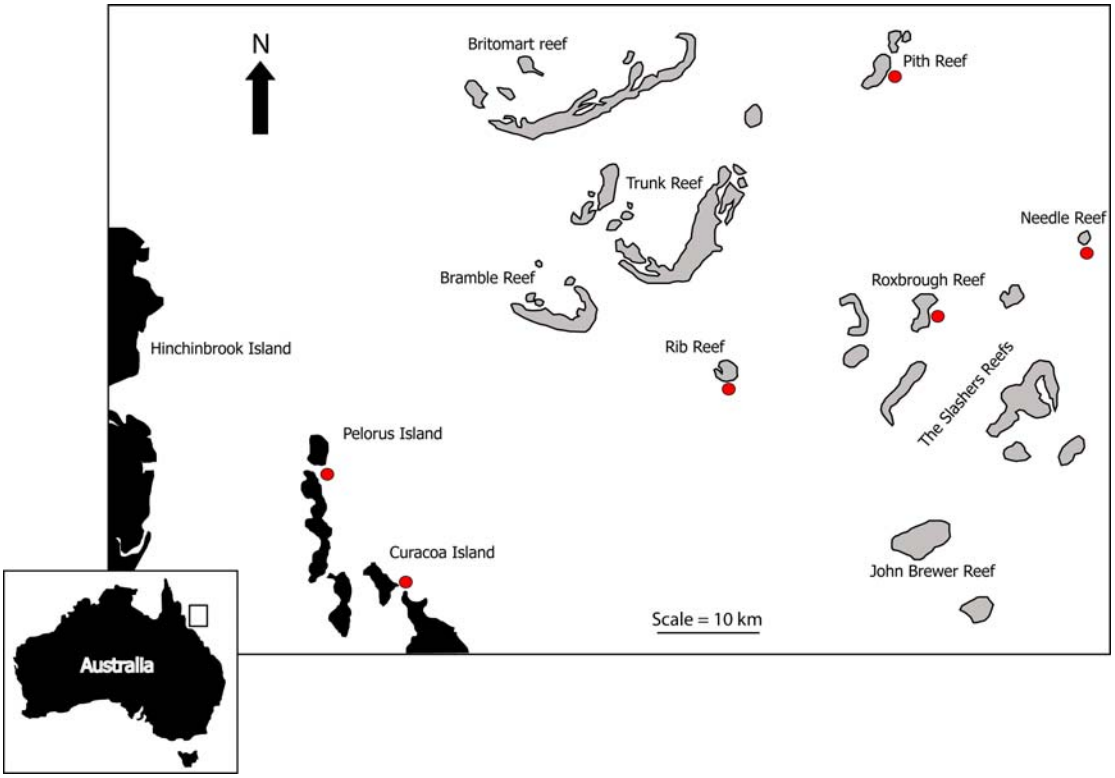

Figure 5.1 Map of reefs across inner-, mid- and outer-shelf locations on the central GBR, Australia. Red circles indicate reefs surveyed at each shelf locations. Black objects represent land grey objects represent reef complexes.

5.2.2 Feeding biology

5.2.2.1 Sample collection

To quantify temporal and spatial retention efficiency patterns of ultraplankton by *R. odorabile* ambient water and water expelled through sponge exhalant canals (oscula) were collected in pairs across reef shelf locations using a structured hierarchical sampling design (Figure 5.2). Water samples were collected during December 2005, January 2006 and February 2006 across inner-, mid- and outer-shelf reef locations. During each month of sampling, water samples were collected at

each shelf location. At each shelf location water samples were collected from two different reefs, and within each reef, water samples were collected randomly from *R. odorabile* at 2 different sites (separated by 150 m) but of similar habitat structure.

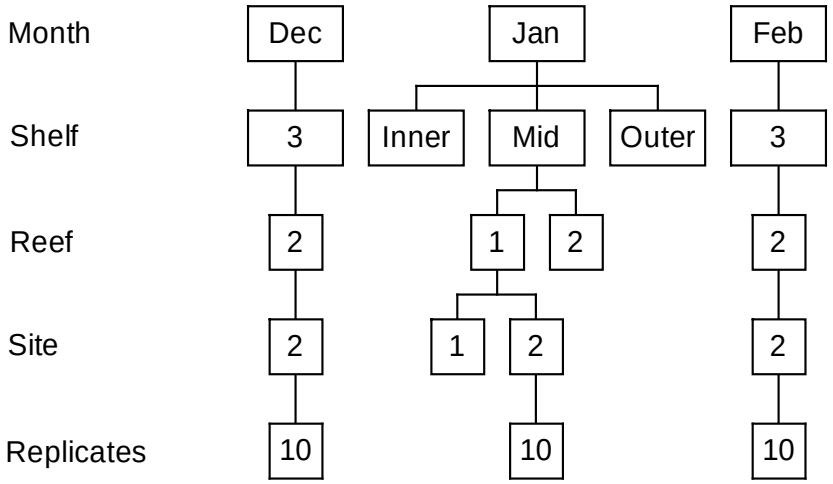

Figure 5.2 Hierarchical sampling design employed to determine spatial and temporal retention efficiency patterns of ultraplankton by *R. odorabile* on the central GBR, Australia.

In situ water sampling was carried out on SCUBA at a depth between 8 and 12 m at inner-mid- and outer-shelf reef locations. Five ml sterile syringes were used to collect 1 ml water samples. Water samples were collected simultaneously in pairs with one ambient water sample collected 5 cm from the inhalant canal (ostia) and one exhalant water sample collected from the exhalant canal (osculum), as described by Pile et al. (1996). During water sample collection care was taken not to suspend bottom sediments as this could compromise the quality of the water samples collected. During December, January and February 10 replicate *R. odorabile* (n=10) were sampled at each site, however, sample losses during the dive and the cracking of several cryo-vials in liquid nitrogen, resulted in a lower sampling effort at several sites (total of n=345, see Table 5.1).

Both ambient and exhalant water samples were placed into separate 1 ml cryo-vials and fixed with paraformaldehyde (0.2% final concentration) for 25 min to preserve the accessory pigments within ultraplankton for flow cytometry analysis (Campbell et al. 1994). All water samples were subsequently frozen in liquid nitrogen and stored at -80°C until analysed using flow-cytometry.

Table 5.1 Sample size for paired inhalant/exhalant water sample collected at each site, during December (2005), January and February (2006).

	Months sampled		
Shelf location	**December**	**January**	**February**
Inner-shelf			
Pelorus Reef 1	10[a] (7)	10[a] (7)	8[a] (8)
Pelorus Reef 2	9[a] (6)	9[a] (8)	10[a] (7)
Curacao Reef 1	10[a] (8)	10[a] (8)	10[a] (6)
Curacao Reef 2	10[a] (7)	10[a] (6)	9[a] (8)
Mid-shelf			
Rib Reef 1	10[a] (6)	10[a] (7)	9[a] (6)
Rib Reef 2	9[a] (6)	10[a] (6)	9[a] (7)
Roxbrough Reef 1	10[a] (7)	9[a] (5)	10[a] (8)
Roxbrough Reef 2	10[a] (6)	10[a] (7)	8[a] (7)
Outer-shelf			
Pith Reef 1	10[a] (8)	10[a] (6)	9[a] (5)
Pith Reef 2	9[a] (7)	8[a] (5)	10[a] (7)
Needle Reef 1	10[a] (8)	10[a] (5)	8[a] (6)
Needle Reef 2	10[a] (7)	8[a] (5)	8[a] (7)

[a]-Number of paired water samples collected minus samples lost during collection and storage in liquid nitrogen. Values in parentheses indicate the actual number of samples that were used for statistical analysis after contaminated samples were removed from the data set (see section 5.2.2.2).

5.2.2.2 Sample preparation and flow cytometry analysis

Samples were prepared for flow cytometry following the methodology of Marie et al. (1997). Water samples were stained with SYBR Green I (DNA specific stain, Molecular Probes Inc. 0561-3), which allows for the analysis of both phytoplankton and bacterial cells simultaneously (Marie et al. 1997). Briefly, water samples were incubated for 30 min in the presence of 0.1 g of RNase A and B (Sigma R-4875) (1:1[wt/wt]) litre^{-1} in a water bath at 37°C. After incubation 100 µl of the water sample was added to a flow tube containing 900 µl of 0.1 µm filtered artificial seawater, 45 µl of

potassium citrate (final concentration, 30 mM) and 5 µl of a 1% solution of SYBR Green I (final concentration, 1 x 10^{-4}), and incubated in the dark at room temperature for 15 min to allow for cell staining (Marie et al. 1997). After staining 5 µl of yellow-green fluorescent beads (0.95 µm diameter; Polysciences Inc) were added to the samples as an internal standard at a final concentration of 5 x 10^3 beads.ml^{-1} just prior to the analysis (Marie et al. 1997).

Water samples were analysed to enumerate ultraplankton retained by *R. odorabile* using a CyAn ADP flow cytometer (Dako Cytomation) at the Molecular Sciences Department at James Cook University, Townsville, following the methodology of Marie et al. (1997). Samples were illuminated with 1 W of the 488 nm line of a 6 W argon laser, and orange, red and green fluorescence were collected through band pass filters at 575, 680 and 530 nm respectively. Samples were run for 1 min periods with an event rate of <1000 events.s^{-1} to prevent coincidence. The five measured parameters, orange fluorescence (from phycoerythrin), red fluorescence (from chl *a*), green fluorescence (from DNA stained with SYBR Green I), forward light scatter (FALS) and right-angle light scatter signals (RALS), were recorded and sorted in list mode on a 4-decade logarithmic scale. Attempts were made to analyse list mode files using CYTOWIN (Vaulot 1989), a custom designed software package commonly used for ultraplankton enumeration, however, list mode files generated from this flow-cytometer were not recognised by CYTOWIN. Therefore, ultraplankton populations were identified and enumerated using WinMDI (Version 2.8, Joseph Trotter) a freeware package also used for the analysis of flowcytometry list mode files. Ultraplankton populations were identified to general cell types of heterotrophic bacteria (Bac), *Prochlorococcus* spp. (Pro), *Synechococcus*-type cyanobacteria (hereafter referred to as *Synechococcus* spp.) (Syn), and picoeukaryotes <3 µm (Pico). To visually confirm the presence of these ultraplankton cell types, except for *Prochlorococcus* spp., the remaining stained water samples were filtered onto 0.2 µm black nucleopore membrane filters and observed using a Leica DMLB epifluorescence microscope

(at 400x magnification). Cell diameters of picoeukaryotes were measured using the image analysis program Leica IM50 Image Manager (n=20, for each shelf location and month sampled, total n=180).

After processing, 106 of the 345 paired feeding samples analysed were noted as contaminated, most likely with sediment, sponge cells or mucus expelled from the sponges, making ultraplankton populations within these water samples unidentifiable. Therefore, to ensure conservative estimates of ultraplankton retention, these contaminated samples were removed from the data set (see Table 5.1 for replicate numbers per site used for the statistical analysis). Subsequently, the retention efficiency of each of the four ultraplankton cell types were calculated as [(ultraplankton cell count ambient water – ultraplankton cell count exhalant water) / ultraplankton cell count ambient water].

5.2.2.3 Carbon and nitrogen retention

To conservatively estimate the retention of carbon and nitrogen in the form of ultraplankton by *R. odorabile* across inner-, mid- and outer-shelf reef locations of the central GBR, the mean number of each ultraplankton cell type retained by *R. odorabile* was converted to μg $C.l^{-1}$ and μg $N.l^{-1}$ using published literature cellular conversions. *R. odorabile* only significantly retained picoeukaryotes <3 μm across shelf locations and between months sampled, with the exception of heterotrophic bacteria during February (see results section 5.3.2.1). Therefore only carbon and nitrogen equivalents were determined for picoeukaryotes <3 μm and heterotrophic bacteria during February. For heterotrophic bacteria, cellular conversions to carbon used were 20 fg C $cell^{-1}$ with a C/N ratio of 3.5 (Wheeler and Kirchman 1986). For picoeukaryotes < 3 μm, cellular conversions to carbon were calculated using pg C $cell^{-1}$ = 0.109 x cell bio-volume $(\mu m^3)^{0.991}$ and conversions to nitrogen were calculated using pg N $cell^{-1}$ = 0.0172 x cell bio-volume $(\mu m^3)^{1.023}$ (Montagnes et al. 1994). A 2-factor ANOVA of the mean bio-volumes of picoeukaryotes <3 μm (as determined from fluorescence microscopy

measurements) identified a shelf x month interaction, resulting in different bio-volumes of picoeukaryotes <3 μm between months and shelf locations (see Appendix 1). During December the mean bio-volumes of picoeukaryotes <3 μm on inner-shelf locations (1.35 ± 0.18 μm^3) differed significantly from mid- (2.64 ± 0.44 μm^3) and outer-shelf locations (2.46 ± 0.60 μm^3). The mean bio-volumes of picoeukaryotes <3 μm did not differ across shelf locations during January (1.52 ± 0.21 μm^3) or February (1.33 + 0.23 μm^3). Estimating carbon and nitrogen content of ultraplankton cell types with cellular conversions is a common method used in many studies to estimate carbon and nitrogen fluxes by suspension feeders in benthic habitats (Pile et al. 1996; Ribes et al. 1999a,b; Pile and Young 2006).

5.2.3 Pumping activity

To determine the daily pumping activity for *R. odorabile* across inner-, mid- and outer-shelf reef locations exhalant current velocities were quantified *in situ* for four individuals at Pelorus Island (inner-shelf), four individuals at Rib Reef (mid-shelf) and four individuals at Pith Reef (outer-shelf) during August and September 2005. For each oscula, exhalant velocities were measured every 5 min for a 24 h period using two heated microthermistor flow-meters equipped with data loggers which recorded all exhalant current flow measurements as a voltage (modified from LaBarbera and Vogel 1976; described in Bell et al. 1999). Temperature data loggers were attached to the underwater housings of each medusa to allow the logged pumping data to be calibrated for temperature variation. Individual microthermistors were placed perpendicular to exhalant current flow (determined by injecting fluorescent dye into the base of individual sponges) and were left in place for 24 h. To convert logged pumping data to flow rates ($cm.s^{-1}$) for each oscula measured, logged voltage data were calibrated with logged temperature data and converted to $cm.s^{-1}$ using calibration coefficients for each microthermistor.

Sponge oscula were photographed prior to the experiment and at the conclusion of the experiment to determine the average cross-sectional area. Oscula area was determined from digitised images using computer imagery software (Leica IM50). To assume accuracy only exhalant velocities from microthermistors found in the centre of each osculum at the end of the experiment were used for data analysis. As a result low sample sizes were obtained during August and September across inner- (n=1, n=2, respectively), mid- (n=1, n=1, respectively), and outer-shelf locations (n=1, n=1, respectively) precluding statistical analysis to be performed. To estimate the mean volume processed ($ml.s^{-1}$) by *R. odorabile* across reef shelf locations it was assumed, as in previous studies, that the exhalant current profile for sponges fits the plug flow model (Pile et al. 1996 and references within). The mean pumping rate for *R. odorabile* was determined using Q = uA, where Q is the pumping rate ($ml.s^{-1}$), u is the exhalant current velocity ($cm.s^{-1}$), and A is the mean cross-sectional area of the oscula (cm^2) (Pile et al. 1996). Although microthermistor equipped data loggers were deployed for 24 h periods in an attempt to measure daily pumping activity of sponges unreliable readings were obtained for several individuals measured. To obtain a conservative estimate of sponge pumping activity these unreliable readings were removed from the data set prior to data analysis.

5.2.4 Statistical analysis

All statistical analyses were performed using SYSTAT version 10. To ensure data met the assumptions of the statistical analyses performed, all data were checked for homogeneity of variance and normality using residuals versus predicted values plots and Q-Q plots of residuals, respectively. Data transformations for each statistical analysis are discussed below. Despite unequal sample sizes for inhalant/exhalant water samples collected across shelf locations and between months sampled, all data sets were analysed using ANOVA. ANOVA is robust and provided the

data meets the assumptions of ANOVA, statistical analysis can still be carried out. However, care should be taken when interpreting the results (Underwood 1981).

To examine differences in the overall abundance of ultraplankton cell types between months and across shelf locations sampled the abundance data for each ultraplankton cell type (heterotrophic bacteria, *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes <3 μm) were analysed together using a 4-factor nested multivariate analysis of variance (MANOVA) model. In addition, each ultraplankton cell type was also analysed separately using univariate 4-factor nested ANOVA models to identify differences in the abundance of each ultraplankton cell type between the 4 factors analysed. (Factors: (1) Month (fixed factor) – 3 months, (2) Shelf (fixed factor) – 3 shelf locations, (3) Reef nested within Month x Shelf (random factor) – 6 reefs, (4) Site nested within reef (random factor) – 2 sites per reef). Abundance data for heterotrophic bacteria, *Prochlorococcus* spp., and *Synechococcus* spp. cell types were log transformed, while the abundance data for picoeukaryotes <3 μm were square root transformed to meet the assumptions of both MANOVA and ANOVA (Underwood 1981; Quinn and Keough 2002).

To examine significant effects of *R. odorabile* on ambient cell concentrations of each ultraplankton cell type (heterotrophic bacteria, *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes <3 μm), data for paired inhalant/exhalant water samples collected at sites and reefs were pooled within each shelf location for each month sampled and analysed using 3-factor blocked ANOVA models (Factors: (1) Shelf (fixed factor) – 3 shelf locations, (2) Individual (blocked factor) – (December – inner- n=28, mid- n=24 and outer-shelf n=30; January – inner- n=29, mid- n=25 and outer-shelf n=21; February – inner- n=29, mid- n=28 and outer-shelf n=25) and (3) Sample – inhalant and exhalant (fixed factor)). All data were log transformed to meet the assumptions of ANOVA (Underwood 1981; Quinn and Keough 2002). Due to multiple testing a Bonferroni transformed experiment-wise $\alpha = 0.0125$ was employed to reduce type I error rates (Quinn and Keough 2002).

Four-factor partially nested ANOVA models were employed to determine significant differences in the retention efficiencies of each ultraplankton cell type (heterotrophic bacteria, *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes <3 μm) by *R. odorabile* across shelf locations and between months sampled. (Factors: (1) Month (fixed factor) – 3 months, (2) Shelf (fixed factor) – 3 shelf locations, (3) Reef nested within Month x Shelf (random factor) – 6 reefs, (4) Site nested within Reef (random factor) – 2 sites per reef). The retention efficiency data for picoeukaryotes <3 μm were arcsin square root transformed to meet the assumptions of ANOVA (Underwood 1981; Quinn and Keough 2002). Due to multiple testing a Bonferroni transformed experiment-wise $\alpha = 0.0125$ was employed to reduce type I error rates (Quinn and Keough 2002).

To determine significant differences in the total number of picoeukaryotes cells <3 μm retained by *R. odorabile* across shelf locations and between months sampled a 4-factor partially nested ANOVA was employed (Factors: (1) Month (fixed factor) – 3 months, (2) Shelf (fixed factor) – 3 shelf locations, (3) Reef nested within Month x Shelf (random factor) – 6 reefs, (4) Site nested within Reef (random factor) – 2 sites per reef). Retention data for picoeukaryotes <3 μm was square root transformed to meet the assumptions of ANOVA (Underwood 1981; Quinn and Keough 2002).

Four-factor partially nested ANOVA models were employed to determine significant differences in the amount of carbon and nitrogen retained per ml of water filtered by *R. odorabile* in the form of picoeukaryotes <3 μm across shelf locations and between months sampled (Factors: (1) Month (fixed factor) – 3 months, (2) Shelf (fixed factor) – 3 shelf locations, (3) Reef nested within Month x Shelf (random factor) – 6 reefs, (4) Site nested within Reef (random factor) – 2 sites per reef). Retention data for both carbon and nitrogen were square root transformed to meet the assumptions of ANOVA (Underwood 1981; Quinn and Keough 2002).

Three-factor nested ANOVA models were employed to determine significant differences in (a) the total number of heterotrophic bacteria retained and (b) the retention of carbon and nitrogen equivalents in the form of heterotrophic bacteria across inner-, mid- and outer-shelf locations during February (Factors: (1) Shelf (fixed factor) – 3 shelf locations, (2) Reef nested within Shelf (random factor) – 2 reefs per shelf, (3) Sites nested within Reef (random factor) – 2 sites per reef). All data met the assumptions of ANOVA and did not require transformations.

5.3 Results

5.3.1 Ultraplankton abundance

The composition of ultraplankton cell types available to *R. odorabile* populations were similar across inner-, mid- and outer-shelf reef locations, consisting of heterotrophic bacteria, *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes <3 μm (Figure 5.3). The abundance of ultraplankton cell types varied between sites sampled highlighting some within reef variation in ultraplankton abundance, presumably driven by differences in microhabitats between sites sampled. The mean abundance of the ultraplankton community available to *R.odorabile* did not differ significantly between months or across reefs sampled (Table 5.2). This was driven by the consistently higher mean abundance of heterotrophic bacteria across shelf locations sampled despite significant variation in the abundance of heterotrophic bacteria between sites (nested within reef) (Figure 5.3, Table 5.3).

Heterotrophic bacteria were the most abundant of the ultraplankton cell types available to *R. odorabile* across inner- (370,365 ± 22,108 cells.ml^{-1}), mid- (393,794 ± 39,367 cells.ml^{-1}) and outer-shelf reefs (356,821 ± 30,842 cells.ml^{-1}). Across shelf locations, heterotrophic bacteria were up to 5, 3 and 21 times more abundant than *Prochlorococcus* spp., *Synechococcus* spp. and

picoeukaryotes <3 μm, respectively. However, the mean abundance of *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes <3 μm varied differentially between shelf locations sampled (Figure 5.3). Picoeukaryotes <3 μm were the least abundant cell type available to *R. odorabile*, however, they were the main cell type significantly retained by *R. odorabile* across shelf locations sampled (see section 5.3.2.2).

The mean abundance of *Prochlorococcus* spp. cell types increased across shelf locations with cells available to *R. odorabile* being twice as abundant on mid- (161,412 ± 14,771 cells.ml^{-1}) and outer-shelf reefs (170,169 ± 65,938 cells.ml^{-1}) than on inner-shelf reefs (77,850 ± 4960 cells.ml^{-1}) (Figure 5.3, Table 5.3). In addition, the mean abundance of *Synechococcus* spp. and picoeukaryotes <3 μm decreased across shelf locations, with *Synechococcus* spp. cell types being 1.3 and 1.7 times more abundant on inner-shelf reefs (209,155 ± 38,133 cells.ml^{-1}) than mid- (156,748 ± 21,049 cells.ml^{-1}) and outer-shelf reefs (122,163 ± 38,742 cells.ml^{-1}), respectively. Most importantly, given that picoeukaryotes <3 μm are the main cell types retained, picoeukaryotes <3 μm were 1.9 and 3.6 times more abundant on inner-shelf reefs (61,049 ± 3,108 cells.ml^{-1}) than mid- (32,421 ± 8,104 cells.ml^{-1}) and outer-shelf reefs (16,742 ± 5,945 cells.ml^{-1}), respectively (Figure 5.3). Furthermore, the abundance of picoeukaryotes <3 μm varied significantly between reefs (nested within months x shelf) and between sites (nested within reefs) sampled (Table 5.3).

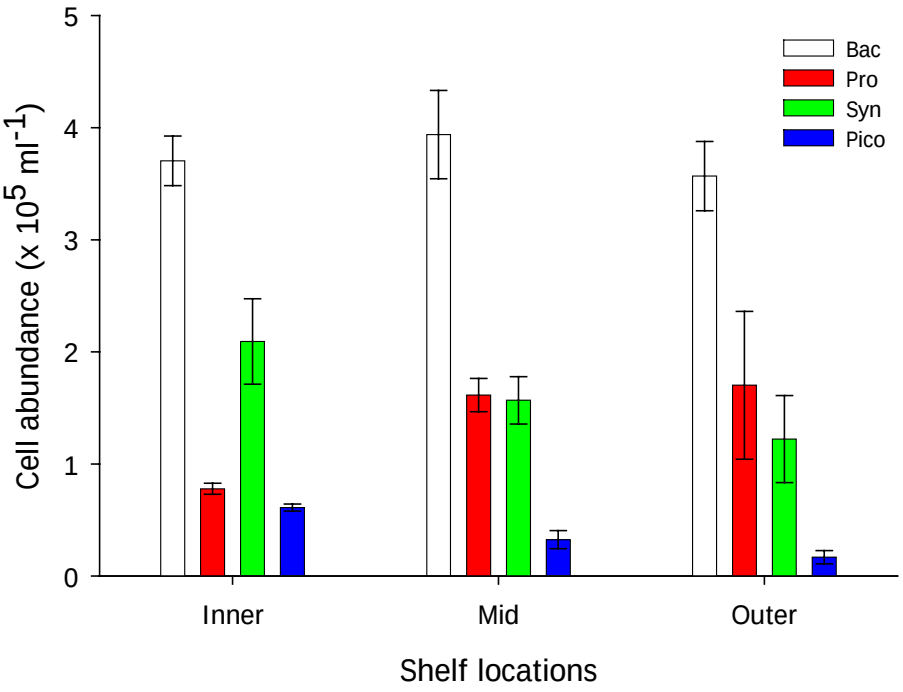

Figure 5.3 Mean cell abundance (x 10^5 ml^{-1}, ± SE) of four ultraplankton cell types within sponge habitats across inner-, mid- and outer-shelf locations of the central GBR, Australia (n=3). Note: Bac – heterotrophic bacteria, Pro – *Prochlorococcus* spp., Syn – *Synechococcus* spp., Pico – picoeukaryotes <3µm.

Table 5.2 Nested MANOVA (Pillai's trace) model comparing ultraplankton abundance across shelf locations and between months sampled. NumDF: numerator degrees of freedom; DenDF: denominator degrees of freedom. (Month: December, January and February; Shelf: Inner-, mid- and outer-shelf; Reef: 2 reefs per shelf location; Site: 2 sites per reef).

Source	**Pillai**	**F**	**NumDF**	**DenDF**	***P***
Month	0.841	1.27	8	14	0.332
Shelf	1.189	2.57	8	14	0.059
Month x Shelf	1.469	1.31	16	36	0.246
Reef (Month x Shelf)	2.703	1.39	36	24	0.201
Site (Reef)	0.323	3.15	24	860	<0.001

Table 5.3 Four-factor nested ANOVA models examining significant differences in the abundance of (a) log transformed heterotrophic bacteria, (b) log transformed *Prochlorococcus* spp., (c) log transformed *Synechococcus* spp. and (d) picoeukaryotes <3 μm in ambient waters surrounding *R. odorabile* across shelf locations and between months on the central GBR. (Month: December 2005, January and February 2006; Shelf: inner-, mid- and outer-shelf locations; Reef: 2 reefs at each shelf location; Sites: 2 sites per reef). Bonferroni corrected $\alpha = 0.05/4 = 0.0125$.

Source	df	MS	F	*P*
(a) Bacteria				
Month	2	0.993	1.62	0.251
Shelf	2	0.053	0.09	0.918
Month x Shelf	4	0.247	0.40	0.802
Reef (Month x Shelf)	9	0.613	0.89	0.582
Site (Reef)	6	0.691	5.45	<0.001
Error	215	0.127		
(b) *Prochlorococcus* spp.				
Month	2	5.665	7.72	0.011
Shelf	2	9.975	13.58	0.002
Month x Shelf	4	2.285	3.11	0.073
Reef (Month x Shelf)	9	0.734	1.48	0.328
Site (Reef)	6	0.498	2.10	0.054
Error	215	0.237		
(c) *Synechococcus* spp.				
Month	2	8.004	9.30	0.006
Shelf	2	6.356	7.38	0.013
Month x Shelf	4	1.490	1.73	0.227
Reef (Month x Shelf)	9	0.861	1.58	0.298
Site (Reef)	6	0.545	2.42	0.028
Error	215	0.225		
(d) Picoeukaryotes				
Month	2	2.091×10^4	0.62	0.559
Shelf	2	3.215×10^5	9.54	0.006
Month x Shelf	4	2.597×10^4	0.77	0.571
Reef (Month x Shelf)	9	3.372×10^4	10.05	0.005
Site (Reef)	6	3.355×10^3	3.04	0.007
Error	215	1.105×10^3		

5.3.2 Retention efficiency of ultraplankton

5.3.2.1 The effect of *Rhopaloeides odorabile* on the ambient cell concentrations of ultraplankton

During December 2005, January and February 2006, *R. odorabile* significantly decreased mean ambient cell concentrations of picoeukaryotes <3 μm across inner-, mid- and outer-shelf locations. However, *R. odorabile* did not significantly affect mean ambient cell concentrations of heterotrophic bacteria, *Prochlorococcus* spp. or *Synechococcus* spp. across shelf locations and

between months sampled, with the exception of heterotrophic bacteria during February 2006 (Table 5.4, Figure 5.4). The significant variation of mean inhalant/exhalant water samples between shelf locations for *Prochlorococcus* spp. and *Synechococcus* spp. was driven by differences in mean cell abundances of inhalant/exhalant water samples between shelf locations. However, mean inhalant/exhalant water samples of heterotrophic bacteria differed significantly between individuals sampled during December 2005 (Table 5.4). This difference between individuals may be explained by the highly sporadic periods of active retention of heterotrophic bacteria by *R. odorabile* sampled within and between reefs across shelf locations (Figure 5.5). Furthermore, sporadic periods of active retention of *Prochlorococcus* spp. and *Synechococcus* spp. may also explain their significant differences in the mean inhalant/exhalant water samples between individuals sampled during February 2006 (Table 5.4).

These results demonstrate that natural environmental gradients have minimal influence on the selection of ultraplankton cell types by *R. odorabile* across shelf locations and between months sampled. However, it is unclear whether the retention efficiencies of ultraplankton remain consistent across temporal and spatial scales.

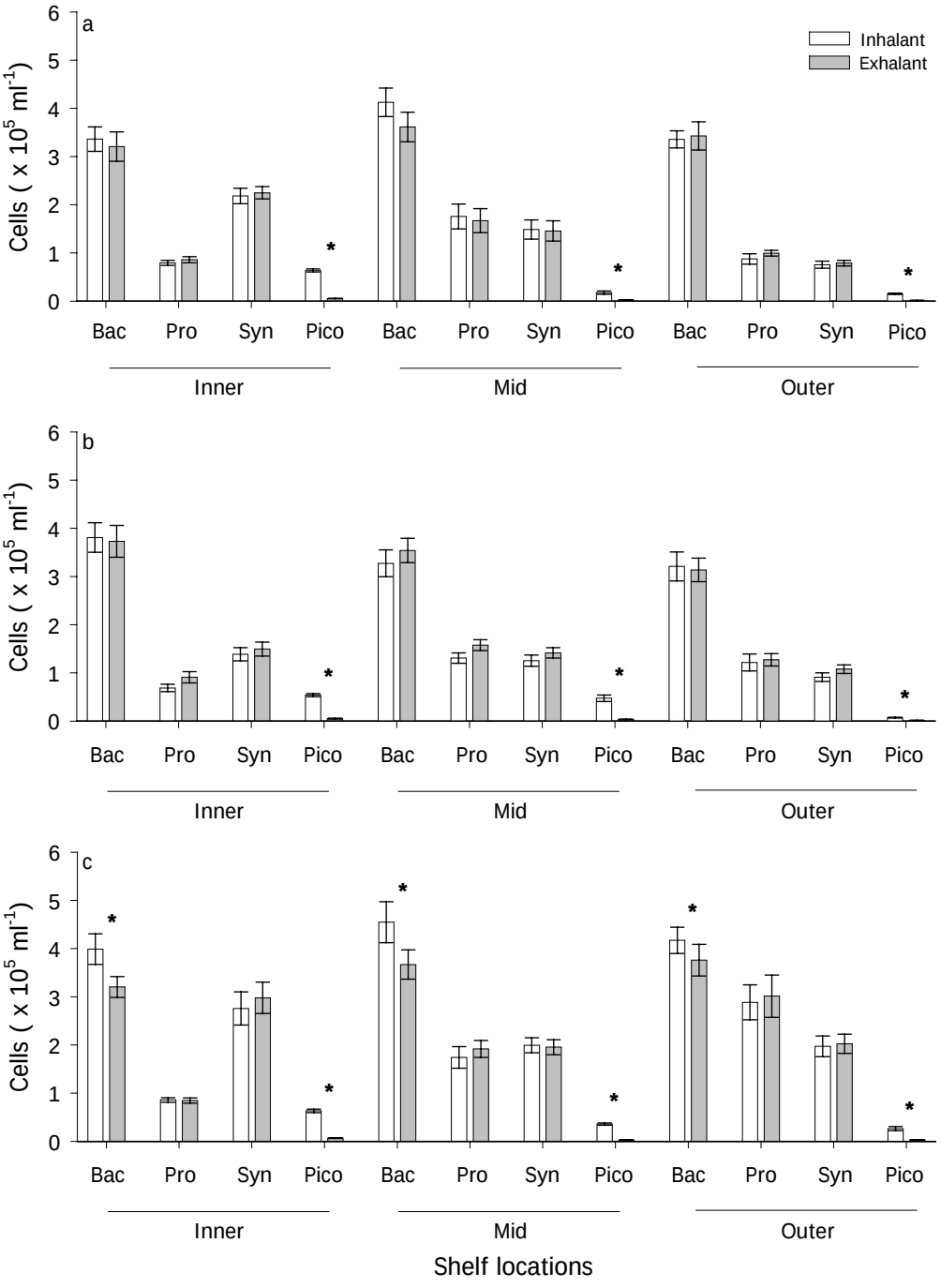

Figure 5.4 Mean cell abundances (x 10^5 ml^{-1}, ± SE) of four ultraplankton cell types for both ambient and exhalant water samples collected from *R. odorabile* during (a) December 2005 (inner – n=28, mid – n=24 and outer – n=30), (b) January 2006 (inner – n=29, mid – n=25 and outer – n=21) and (c) February 2006 (inner – n=29, mid – n=28 and outer – n=25) across shelf locations of the central GBR, Australia. * denotes significant differences between inhalant/exhalant water sample pairs at $P = 0.0125$. Bac – heterotrophic bacteria, Pro – *Prochlorococcus* spp., Syn – *Synechococcus* spp. and Pico – picoeukaryotes <3 μm.

Table 5.4 Blocked ANOVA models to determine the effect of *R. odorabile* on the ambient cell concentrations of (a) heterotrophic bacteria, (b) *Prochlorococcus* spp., (c) *Synechococcus* spp. and (d) picoeukaryotes < 3 μm, across shelf locations of the central GBR during December, January and February. (Shelf: inner-, mid- and outer-shelf; Individuals: December (inner – [n=28], mid – [n=24] and outer – [n=30]), January (inner – [n=29], mid – [n=25] and outer – [n=21]), February (inner – [n=29], mid – [n=28] and outer – [n=25]); Sample: Inhalant and exhalant water samples). Bonferroni corrected $\alpha = 0.05/4 = 0.0125$.

Months		**December**				**January**				**February**		
Source	**df**	**MS**	**F**	***P***	**df**	**MS**	**F**	***P***	**df**	**MS**	**F**	***P***
(a) Bacteria												
Shelf	2	0.274	1.981	0.142	2	0.149	0.903	0.408	2	0.274	1.645	0.197
Individual	29	0.382	2.761	<0.001	28	0.147	0.888	0.629	28	0.226	1.356	0.130
Sample	1	0.400	2.896	0.091	1	0.005	0.030	0.863	1	1.158	6.953	0.009
Shelf x Sample	2	0.048	0.350	0.706	2	0.074	0.451	0.638	2	0.015	0.091	0.913
Error	129	0.138			116	0.165			130	0.167		
(b) *Prochlorococcus* spp.												
Shelf	2	3.600	13.460	<0.001	2	8.285	36.871	<0.001	2	16.169	85.469	<0.001
Individual	29	0.390	1.459	0.080	28	0.394	1.755	0.020	28	0.527	2.786	<0.001
Sample	1	0.127	0.473	0.493	1	1.323	5.886	0.017	1	0.053	0.281	0.597
Shelf x Sample	2	0.240	0.898	0.410	2	0.052	0.231	0.794	2	0.086	0.452	0.637
Error	129	0.267			116	0.225			130	0.189		
(c) *Synechococcus* spp.												
Shelf	2	16.737	61.105	<0.001	2	1.018	4.997	0.008	2	1.030	4.347	0.015
Individual	29	0.390	1.428	0.092	28	0.285	1.398	0.112	28	0.751	3.169	<0.001
Sample	1	0.004	0.013	0.909	1	0.623	3.057	0.083	1	0.012	0.051	0.822
Shelf x Sample	2	0.126	0.458	0.633	2	0.044	0.217	0.806	2	0.129	0.543	0.582
Error	129	0.274			116	0.204			130	0.237		
(d) Picoeukaryotes												
Shelf	2	37.429	100.422	<0.001	2	27.929	72.502	<0.001	2	15.369	41.349	<0.001
Individual	29	0.420	0.420	0.316	28	0.424	1.099	0.352	28	0.286	0.770	0.786
Sample	1	221.373	593.948	<0.001	1	116.230	431.524	<0.001	1	207.741	558.898	<0.001
Shelf x Sample	2	2.685	7.203	0.001	2	3.312	8.598	<0.001	2	1.776	4.777	0.010
Error	129	0.373			116	0.385			130	0.372		

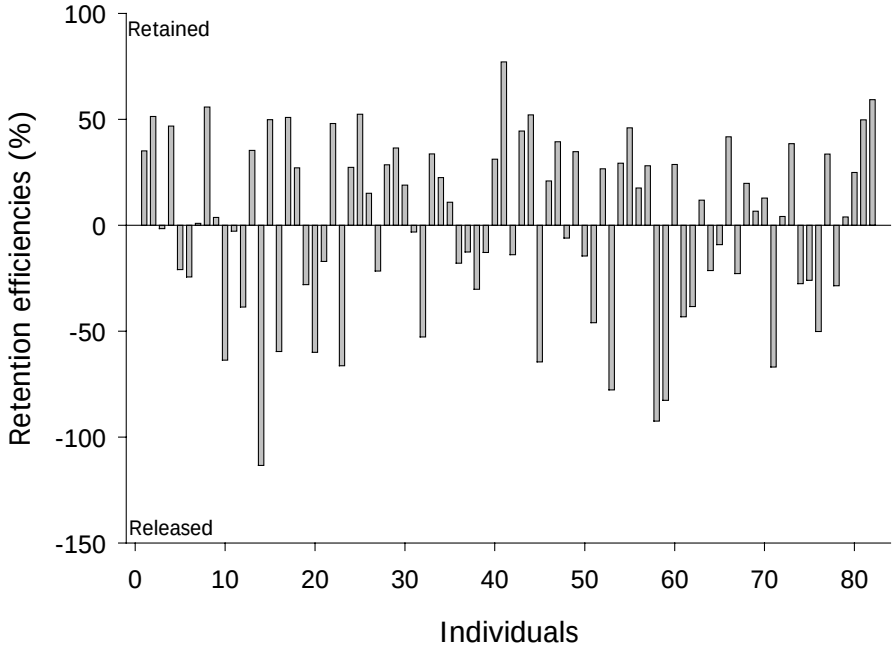

Figure 5.5 Retention efficiencies (%) of heterotrophic bacteria by *R. odorabile* (n=82) across shelf locations of the central GBR, Australia, sampled during December 2005.

5.3.2.2 Retention efficiencies of ultraplankton across temporal and spatial scales

The retention efficiency of each ultraplankton cell type remained consistent across shelf locations and between months sampled. Despite picoeukaryotes <3 μm being the least abundant cell type available across shelf locations, *R. odorabile* retained picoeukaryotes with similar high mean efficiencies across shelf locations (inner-shelf: 90.3 ± 0.7%; mid-shelf: 84.1 ± 4.3%; and outer-shelf: 81.3 ± 5.8%) and between months sampled (Figure 5.6 and Table 5.5). However, heterotrophic bacteria, *Prochlorococcus* spp. and *Synechococcus* spp. were retained and released by *R. odorabile* with consistently lower mean efficiencies than picoeukaryotes <3 μm across shelf locations and between months sampled (Figure 5.6 and Table 5.5).

With the exception of heterotrophic bacteria during February, *R. odorabile* did not significantly affect ambient cell concentrations of heterotrophic bacteria, *Prochlorococcus* spp., or

Synechococcus spp. across shelf locations and between months sampled (Table 5.4). This suggests, with the exception of heterotrophic bacteria during February which was retained with efficiencies of 10.6 ± 7.4% (inner-shelf, mean ± SD), 8.9 ± 6.2% (mid-shelf, mean ± SD) and 3.4 ± 10.1% (outer-shelf, mean ± SD), that these three cell types do not contribute to the diet of *R. odorabile* across shelf locations and between months sampled.

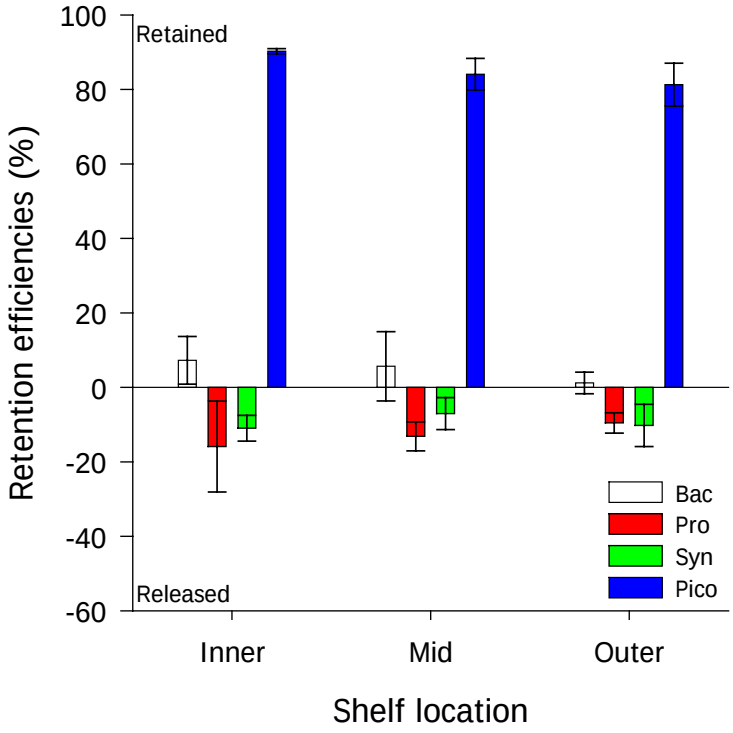

Figure 5.6 Mean retention efficiencies (%, ± SE) of four ultraplankton cell types retained by *R. odorabile* across inner- (n=3), mid- (n=3) and outer-shelf reef locations (n=3) on the central GBR, Australia. Bac – heterotrophic bacteria, Pro – *Prochlorococcus* spp., Syn – *Synechococcus* spp. and Pico – picoeukaryotes <3 μm.

Table 5.5 Four-factor partially nested ANOVA models to examine significant differences in the retention efficiency of arcsin square root trasnformed (a) heterotrophic bacteria, (b) *Prochlorococcus* spp., (c) *Synechococcus* spp. and (d) picoeukaryotes <3 μm by *R. odorabile* across shelf locations of the central GBR, sampled during three different months. (Month: December 2005, January and February 2006; Shelf: inner-, mid- and outer-shelf locations; Reef: 2 reefs at each shelf location; Sites: 2 sites per reef). Bonferroni corrected $\alpha = 0.05/4 = 0.0125$.

Source	df	MS	F	*P*
(a) Bacteria				
Month	2	0.857	5.02	0.034
Shelf	2	0.064	0.37	0.699
Month x Shelf	4	0.060	0.35	0.836
Reef (Month x Shelf)	9	0.171	5.85	0.022
Site (Reef)	6	0.029	0.14	0.990
Error	215	0.024		
(b) *Prochlorococcus* spp.				
Month	2	0.850	4.24	0.051
Shelf	2	0.002	0.01	0.991
Month x Shelf	4	0.541	2.70	0.100
Reef (Month x Shelf)	9	0.201	0.38	0.909
Site (Reef)	6	0.532	1.51	0.177
Error	215	0.353		
(c) *Synechococcus* spp.				
Month	2	0.365	0.72	0.512
Shelf	2	0.236	0.47	0.642
Month x Shelf	4	0.131	0.73	0.897
Reef (Month x Shelf)	9	0.505	0.73	0.681
Site (Reef)	6	0.696	1.65	0.135
Error	215	0.422		
(d) Picoeukaryotes				
Month	2	0.095	0.49	0.628
Shelf	2	0.297	1.53	0.268
Month x Shelf	4	0.314	1.62	0.253
Reef (Month x Shelf)	9	0.194	4.12	0.050
Site (Reef)	6	0.047	2.81	0.012
Error	215	0.017		

5.3.3 Total cells retained

Despite the similar retention efficiency of picoeukaryotes <3 μm across shelf locations and between reefs sampled (Figure 5.6) the total mean number of picoeukaryotes retained by *R. odorabile* differed significantly across shelf locations sampled (Table 5.6 and Table 5.7). *R. odorabile* on

inner-shelf locations (55323 ± 2862 cells.ml^{-1}) retained almost twice as many picoeukaryotes <3 μm than on mid-shelf locations (29602 ± 7761 cells.ml^{-1}) and almost four times as many picoeukaryotes <3 μm than on outer-shelf locations (14269 ± 5038 cells.ml^{-1}) (Table 5.6). The total mean number of picoeukaryotes <3 μm retained by *R. odorabile* also differed significantly between reefs (nested within Month x Shelf) sampled over time (Table 5.7), with the highest number of picoeukaryotes <3 μm being retained at Rib Reef during January (71161 ± 9618 cells.ml^{-1}), whilst the lowest number of picoeukaryotes <3 μm were retained at Needle Reef during January (2460 ± 1033 cells.ml^{-1}) (Figure 5.7). These differences are attributed to between reef and site variation in the abundance of picoeukaryotes <3 μm and their retention by *R. odorabile* across shelf locations (Table 5.3 and Table 5.5) In addition, *R. odorabile* also retained heterotrophic bacteria during February with low but highly variable efficiencies across shelf locations. However, the total mean number of heterotrophic bacteria retained during February did not differ significantly across inner- (42966 ± 29995 cells.ml^{-1}, mean ± SD), mid- (40470 ± 28193 cells.ml^{-1} mean ± SD) and outer-shelf locations (13850 ± 41142 cells.ml^{-1} mean ± SD) (Table 5.8).

Table 5.6 Mean total picoeukaryotes <3 μm retained (± SE) by *R. odorabile* across inner-, mid- and outer-shelf locations of the central GBR. Included are conservative estimates of carbon and nitrogen equivalents for picoeukaryotes <3 μm based on literature cellular conversion values.

Shelf location	Cells retained (Cells 10^4 ml^{-1})	C retained (μg C l^{-1})	N retained (μg N l^{-1})
Inner-shelf	5.53 ± 0.29^{a}	8.38 ± 0.10^{r}	1.34 ± 0.02^{x}
Mid-shelf	2.96 ± 0.78^{b}	5.23 ± 0.84^{s}	0.84 ± 0.13^{y}
Outer-shelf	1.43 ± 0.51^{c}	2.71 ± 0.90^{t}	0.44 ± 0.15^{z}

Superscripts (a, b, c), (r, s, t), and (x, y, z) denote significant differences at $P = 0.05$ as determined by Tukey's HSD multiple comparisons test for square root transformed data.

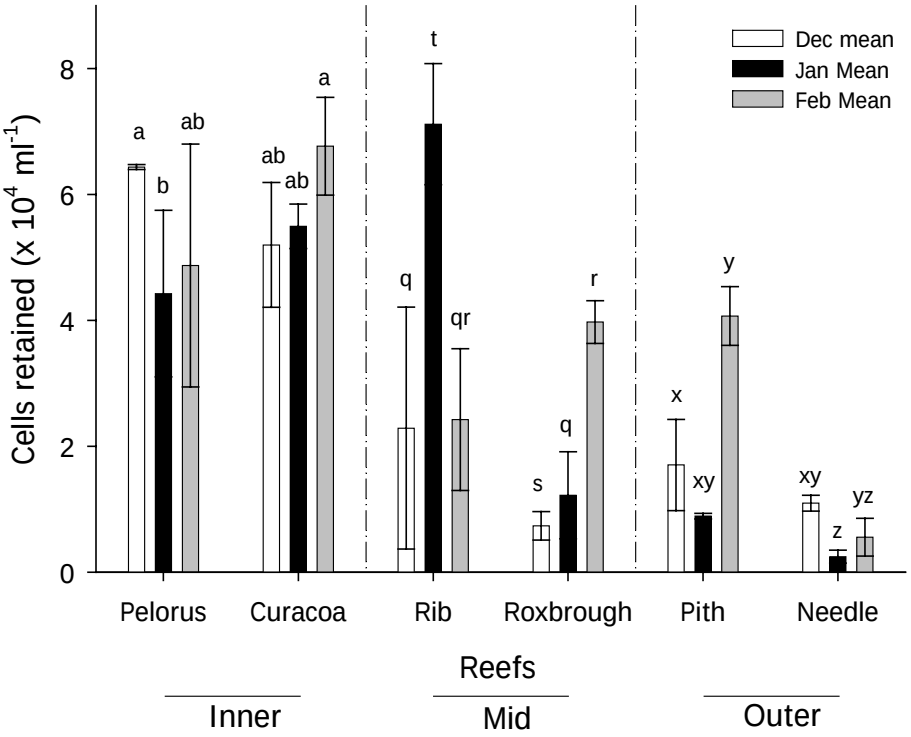

Figure 5.7 Mean total picoeukaryotes retained (x 10^4 ml^{-1}, ± SE) by *R. odorabile* between different reefs across shelf locations of the central GBR, Australia sampled during December, January and February. Superscripts for reefs within inner- (a and b), mid- (q, r, s, t) and outer-shelf locations (x, y, z) denote significant differences (Tukey's HSD multiple comparisons test, P <0.05 for square root transformed data)

Table 5.7 Four-factor partially nested ANOVA model examining differences on the mean total number of picoeukaryotes <3 μm retained by *R. odorabile* across shelf locations of the central GBR, sampled during three different months (Month: December, January and February; Shelf: Inner-, mid- and outer-shelf locations; Reef: 2 reefs at each shelf location; Sites: 2 sites per reef).

Source	df	MS	F	*P*
Picoeukaryotes				
Month	2	1.763 x 10^4	0.48	0.633
Shelf	2	3.168 x 10^5	8.65	0.008
Month x Shelf	4	2.720 x 10^4	0.74	0.587
Reef (Month x Shelf)	9	3.663 x 10^4	10.43	0.005
Site (Reef)	6	3.512 x 10^3	2.97	0.008
Error	215	1.185 x 10^3		

Table 5.8 Three-factor nested ANOVA models examining differences in the retention of (a) heterotrophic bacteria and subsequently its equivalent (b) carbon and (c) nitrogen by *R. odorabile* across inner-, mid- and outer-shelf locations of the central GBR, sampled during December 2005 (Shelf: Inner-, mid- and outer-shelf locations; Reefs: 2 reefs at each shelf location; Site: 2 sites per reef).

Source	df	MS	F	*P*
(a) Total bacteria cells				
Shelf	2	1.733×10^{10}	0.82	0.520
Reef (Shelf)	3	2.114×10^{10}	1.30	0.356
Site (Reef)	6	1.621×10^{10}	0.50	0.811
Error	70	3.280×10^{10}		
(b) Carbon				
Shelf	2	6.945	0.82	0.520
Reef (Shelf)	3	8.461	1.30	0.357
Site (Reef)	6	6.492	0.50	0.810
Error	70	13.124		
(c) Nitrogen				
Shelf	2	0.564	0.82	0.521
Reef (Shelf)	3	0.690	1.30	0.356
Site (Reef)	6	0.529	0.50	0.811
Error	70	1.071		

5.3.4 Carbon and nitrogen retained

Variation in the abundance and retention of picoeukaryotes <3 μm, resulted in significant differences in the retention of carbon and nitrogen by *R. odorabile* across shelf locations and between reefs (nested within month x shelf) sampled (Table 5.9). On inner-shelf reef locations *R. odorabile* significantly retaining 1.6 times more carbon (8.38 ± 0.10 μg $C.l^{-1}$) and nitrogen (1.34 ± 0.02 μg $N.l^{-1}$) in the form of picoeukaryotes <3 μm than individuals on mid-shelf locations (5.23 ± 0.84 μg $C.l^{-1}$ and 0.84 ± 0.13 μg $N.l^{-1}$, respectively) and 3 times more carbon and nitrogen than on outer-shelf locations (2.71 ± 0.90 μg $C.l^{-1}$ and 0.44 ± 0.15 μg $N.l^{-1}$, respectively) (Table 5.6). The mean retention of carbon and nitrogen varied significantly between reefs (nested within month x shelf) sampled (Table 5.9) with the highest retention of carbon (11.7 ± 1.59 μg $C.l^{-1}$) and nitrogen (1.88 ± 0.26 μg $N.l^{-1}$) being at Rib Reef during January and the lowest retention of carbon (0.41 ± 0.17 μg $C.l^{-1}$) and nitrogen (0.07 ± 0.03 μg $N.l^{-1}$) at Needle Reef during January. In addition, during

February, *R. odorabile* also retained carbon and nitrogen in the form of heterotrophic bacteria. Although not significantly different *R. odorabile* retained almost 3 times more carbon and nitrogen in the form of heterotrophic bacteria on inner- (0.86 ± 0.60 μg $C.l^{-1}$ and 0.25 ± 0.17 μg $N.l^{-1}$) and mid-shelf reefs (0.81 ± 0.56 μg $C.l^{-1}$ and 0.23 ± 0.16 μg $N.l^{-1}$) than on inner-shelf reefs (0.28 ± 0.82 μg $C.l^{-1}$ and 0.08 ± 0.24 μg $N.l^{-1}$) (Table 5.8).

Table 5.9 Four-factor partially nested ANOVA models examining differences in the retention of (a) carbon and (b) nitrogen by *R. odorabile* across shelf locations of the central GBR, sampled during three different months (Month: December, January and February; Shelf: Inner-, mid- and outer-shelf locations; Reef: 2 reefs at each shelf location; Sites: 2 sites per reef).

Source	df	MS	F	*P*
(a) Carbon				
Month	2	0.771	0.13	0.880
Shelf	2	37.853	6.35	0.019
Month x Shelf	4	3.792	0.64	0.650
Reef (Month x Shelf)	9	5.967	6.42	0.017
Site (Reef)	6	0.930	4.29	<0.001
Error	215	0.217		
(b) Nitrogen				
Month	2	0.129	0.14	0.875
Shelf	2	5.989	6.27	0.020
Month x Shelf	4	0.688	0.64	0.649
Reef (Month x Shelf)	9	0.955	6.32	0.018
Site (Reef)	6	0.151	4.34	<0.001
Error	215	0.035		

5.3.5 Pumping activity

The exhalant current velocities of individual osculum for *R. odorabile* ranged between 0.02 and 31.90 $cm.s^{-1}$ across shelf locations of the central GBR during August and September 2005. This equated to mean pumping velocities per osculum of 5.02 ± 2.44 $cm.s^{-1}$at Pelorus Island (n=3), 4.02 ± 0.08 and 9.49 ± 0.15 $cm.s^{-1}$ for the two *R. odorabile* at Rib Reef (averaged over the recorded time period for each individual) and 15.14 ± 0.24 and 25.62 ± 0.35 $cm.s^{-1}$ for the two *R. odorabile* at Pith Reef (averaged over the recorded time period for each individual). With mean oscula diameters of

1.22 ± 0.02 cm^2 at Pelorus Island (n=3), 0.10 and 0.13 cm^2 for the two *R. odorabile* at Rib Reef, and 0.08 and 0.05 cm^2 for the two *R. odorabile* at Pith Reef, sponge pumping rates ranged between 0.01 and 3.01 $ml.s^{-1}$ per osculum across shelf locations of the central GBR (Figure 5.8). This resulted in mean pumping rates per osculum of 0.55 ± 0.21 $ml.s^{-1}$ at Pelorus Island (n=3), 0.39 ± 0.01 and 1.25 ± 0.02 $ml.s^{-1}$ for the two *R. odorabile* measured at Rib Reef and 1.26 ± 0.02 and 1.26 ± 0.01 $ml.s^{-1}$for the two *R. odorabile* measured at Pith Reef. The pumping activity of *R. odorabile* varied over time across shelf locations of the central GBR (Figure 5.8). Two of the three individuals at Pelorus Island displayed periods of inactivity, while one *R. odorabile* at Pelorus Island had erratic pumping behaviour late in the afternoon and early morning (Figure 5.8). In contrast, pumping activity patterns of *R. odorabile* at Rib Reef and Pith Reef remained relatively consistent over time. However, at least one individual at Rib Reef and Pith Reef exhibited periods of inactivity over the time period measured (Figure 5.8). Overall, there appears to be no consistent pattern of pumping activity for sponges sampled at Pelorus Island (inner-shelf), Rib Reef (mid-shelf) or Pith Reef (outer-shelf). However, low replication at each of the reefs sampled precludes an accurate description of temporal and spatial variation in patterns of pumping activity.

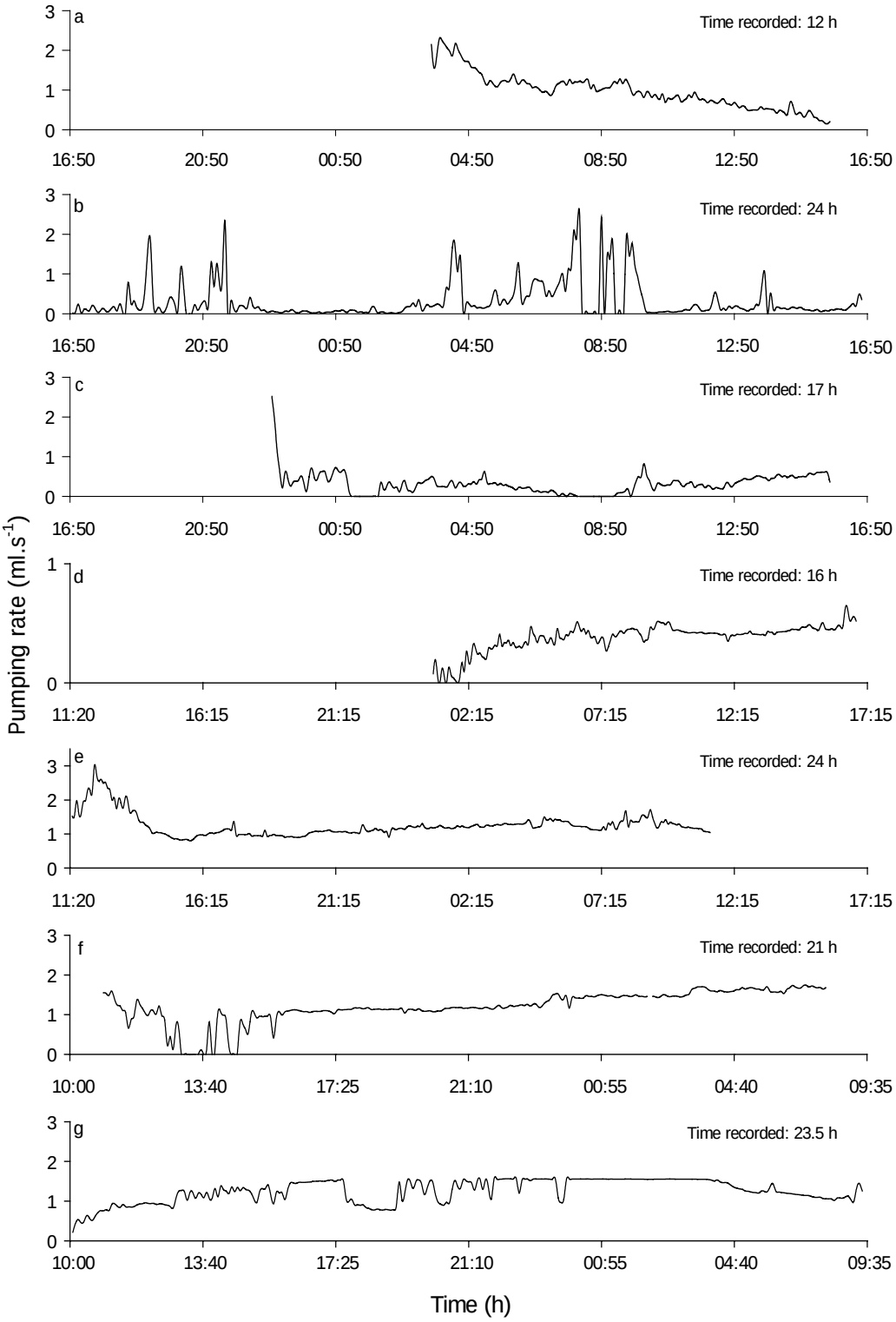

Figure 5.8 Daily pumping activity (ml.s^{-1}) of *R. odorabile* across inner-, mid- and outer-shelf locations of the central GBR during August and September 2005 (a) Pelorus Island – August, individual 1, (b) Pelorus Island – September, individual 1, (c) Pelorus Island – September, individual 2, (d) Rib Reef – August, individual 1, (e) Rib Reef – September, individual 1, (f) Pith Reef – August, individual 1, (g) Pith Reef – September, individual 1.

5.4 Discussion

This study measured, for the first time, *in situ* spatial and temporal retention efficiencies of ultraplankton <3 μm by the common coral reef demosponge *R. odorabile* living in contrasting habitats across a distinct environmental gradient. The outcome of this approach is that the composition of ultraplankton cell types available to *R. odorabile* was consistent across shelf locations and between months. However, the mean abundance of *Prochlorococcus* spp., *Synechococcus* spp., and picoeukaryotes <3 μm available to *R. odorabile* differed strongly across shelf locations. The abundance of picoeukaryotes <3 μm were almost 1.5 and 3 times more abundant on inner-shelf reefs than mid- and outer-shelf reefs, and these were the main cell types retained across shelf locations. However, *R. odorabile* also retained small percentages of heterotrophic bacteria during February. Regardless of shelf location occupied or the abundance of picoeukaryotes <3 μm *R. odorabile* retains picoeukaryotes <3 μm with similar efficiencies irrespective of month sampled. However, decreased cell abundance of picoeukaryotes <3 μm across shelf locations towards the outer-shelf reef resulted in *R. odorabile* retaining almost 2 and 3 times more carbon and nitrogen per litre of water filtered (in the form of picoeukaryotes <3 μm) on inner-shelf reef locations, compared to mid- and outer-shelf reef locations, respectively.

The ultraplankton community quantified across shelf locations of the central GBR during this study is typical of other coral reefs within the GBR region (Furnas and Mitchell 1986; Ayukai 1995; Pile 2005). Despite the consistent abundance of the ultraplankton community across shelf locations, and between months sampled as a whole, the mean abundance of *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes <3 μm differed between shelf locations sampled. In tropical areas *Prochlorococcus* spp. are generally more abundant in oligotrophic waters and low in abundance in eutrophic areas (reviewed in Partensky et al. 1999). During this study the abundance of *Prochlorococcus* spp. followed a similar trend with higher abundances on offshore oligotrophic

reefs than on inshore eutrophic reefs. In contrast to *Prochlorococcus* spp., *Synechococcus* spp. preferentially thrive in areas enriched with nutrients by coastal inputs and strong upwelling (reviewed in Partensky et al. 1999). This was clearly the case during this study where cell abundance patterns of *Synechococcus* spp. decrease with increasing distance from the coastline. Furthermore, a 3 to 4 fold increase in nitrogen loads and the 5 to 10 fold increase in phosphorous in inner-shelf waters compared with outer-shelf waters (McKergow et al. 2005) is suggested to be responsible for cross-shelf phytoplankton biomass and community dynamics (Furnas and Mitchell 1986; Brodie et al. 1997; Cooper et al. 2007). This may explain why the abundance of picoeukaryotes <3 μm were almost 4 times more abundant on inner-shelf compared to outer-shelf reef locations.

This study clearly identifies that irrespective of months sampled *R. odorabile* selectively retains picoeukaryotes <3 μm with similar high efficiencies (up to 92%) across shelf locations of the central GBR. These results contradict the initial hypothesis of this study that *R. odorabile*, like other shallow water demosponges, would unselectively retain a range of ultraplankton cell types (Resiwig 1971a; Pile et al. 1996; Ribes et al. 1999a). Variation in the retention of either ultraplankton, phytoplankton and dissolved or detrital organic carbon in proportion to their availability has been identified for a number of different sessile filter feeders (Pile et al. 1996; Ribes et al. 1998, Bell et al. 1999; Ribes et al. 1999a,b), supporting the theory that prey items are retained proportionally to their availability for many species (Hughes 1980; Coma et al. 2001). However, *R. odorabile* are not opportunistic feeders and selectively retain picoeukaryotes <3 μm with high efficiencies across shelf locations, despite picoeukaryotes <3 μm being almost 2 and 4 times more abundant on inner-shelf reefs compared to mid- and outer-shelf reefs, respectively. While the results presented indicate stable feeding patterns between months sampled they do not infer that feeding patterns remain stable between different seasons. Given *R. odorabile* has a distinct reproductive season (Whalan et

al. 2007b) it is plausible that the selection and retention efficiencies of ultraplankton cell types may change seasonally to optimise energy acquisition, which has previously been identified for the temperate sponge *Dysidea avara* (Ribes et al. 1999a). However, additional sampling is necessary to elucidate seasonal feeding patterns for *R. odorabile*.

Selective feeding by sponges is a controversial issue and there is a growing debate within the literature as to whether sponges are selective feeders or not (Riisgard and Larsen 2001; Pile et al. 1996; Yahel et al. 2006). Although sponges have been considered non-selective filter feeders retaining all particles <50 μm (Bergquist 1978) recent studies have identified both non-selective and selective retention of ultraplankton by different species of sponges in temperate (Pile et al. 1996; Bell et al. 1999; Ribes et al.1999a; Pile 2005), tropical (Reiswig 1971a; Pile 1997, 1999, 2005) and deep-sea habitats (Pile and Young 2006; Yahel et al. 2006). Despite, differences in selective and non-selective retention of ultraplankton by different species of sponges, mechanisms of selectivity may drive partitioning of resources between co-occurring sponges (Thurber 2007) and other suspension and filter feeders in marine benthic communities (Pile 1999, 2005). For example, Pile (1999) identified selective retention of ultraplankton by two of three coral reef sponges living in the same habitat. *Callyspongia vaginalis* and *Spongia tubulifera* selectively retained *Synechococcus*-type cyanobacteria, while *Aplysina fistularis* retained a variety of ultraplankton cell types (Pile 1999). Furthermore, fatty acid analysis of sponge tissues confirmed selective feeding and niche separation between the co-occurring Antarctic sponges *Isodictya setifera*, *Homaxinella balfourensis* and *Kirkpatrickia variolosa* (Thurber 2007). *I. setifera* fed primarily on bacteria, *H. balfourensi* consumed primarily flagellates and larger particles, while *K. variolosa* fed on a mixture of bacteria, flagellates and larger particles. In addition, Pile (2005) found that three sponges (*Dendrilla rosea*, *Aplysilla sulphurea* and an unidentified sponge - SP46) and three ascidians (*Pyura* sp., *Halocynthia* sp. and *Polycarpa pedunculata*), co-occupying the same habitat within temperate ecosystems, utilised different ultraplankton cell types. The three sponge species primarily retained heterotrophic

bacteria while the three species of ascidians retained picoeukaryotes <3 μm (Pile 2005). These studies and the results from this study highlight the importance of selective feeding by sponges as a potential mechanism in maintaining diversity in marine benthic communities through niche separation.

Although *R. odorabile* selectively retains picoeukaryotes across shelf locations and between months sampled, the methods used in this study do not unequivocally eliminate the retention of heterotrophic bacteria by *R. odorabile*. Despite flow cytometry being a powerful tool for quantifying the retention of ultraplankton populations by sponges (Pile et al. 1996) it only measures the total number of cells of heterotrophic bacteria retained and does not discriminate between different bacterial species. These limitations make it difficult to fully resolve the diet of sponges. Duckworth et al. (2006) demonstrated using molecular tools that the species composition of bacteria in exhalant current water was markedly different from inhalant current water for *Aplysina lacunose*, *Callyspongia vaginalis* and *Niphates digitalis*. They proposed that the additional bacterial species in the exhalant current water were microbial symbionts from the sponges, being periodically released into the exhalant current water (Duckworth et al. 2006). *R. odorabile* possess an abundant and diverse bacterial community (with bacterial abundances exceeding approximately 1.0 x 10^{10} bacteria.cm^{-3} of sponge tissue) dominated primarily by a α-proteobacterium that is not present in ambient waters surrounding the sponges (Webster and Hill 2001; Webster et al. 2001a,b). *R. odorabile* may regulate this symbiotic bacterial community by regularly or periodically expelling excessive bacteria into exhalant current waters, a mechanism proposed to control microbial symbiont populations in sponges (Wilkinson 1992). This proposed mechanism may explain the irregular retention/release of heterotrophic bacteria by *R.odorabile* individuals across shelf locations during December 2005 and may account for the low retention of heterotrophic bacteria across shelf locations. Therefore, to determine whether *R. odorabile* retains heterotrophic bacteria or specific

components of the heterotrophic bacterial community requires additional molecular studies of both inhalant and exhalant water samples (Duckworth et al. 2006).

Benthic pelagic coupling by filter feeding invertebrates is a key process in the energetic budget of reef communities (Gili and Coma 1998; Richter and Wunsch 1999; Ribes et al. 2003, 2005; de Goeij et al. 2008) with the retention of ultraplankton contributing to high proportions of ingested carbon and nitrogen by different sessile filter feeding invertebrates (Pile et al. 2006; Gili and Coma 1998; Ribes et al. 1999a,b; Coma et al. 2001; Pile and Young 2006). For some sponges feeding on a diverse assemblage of ultraplankton cell types provides them with a major source of particulate carbon and nitrogen that may be necessary to meet metabolic requirements (Reiswig 1971a; Pile et al. 1996; Ribes et al. 1999a; Pile and Young 2006; Yahel et al. 2007). However, sponges can also obtain carbon from other sources including viruses (Hadas et al. 2006), dissolved organic carbon (DOC) (Reiswig 1990; Yahel et al. 2003; de Goeij et al. 2008; van Duyl et al. 2008) and carbon assimilated from photosynthetic symbionts (Wilkinson 1979, 1983). For *R. odorabile*, the retention of carbon (in the form of picoeukaryotes <3 μm and a small percentage from heterotrophic bacteria) during this study (ranging between 3.0 and 9.3 μg $C.l^{-1}$) is similar to the tropical sponge *Ircinia felix* (7.0 μg $C.l^{-1}$), which is also a poor consumer of ultraplankton (Table 5.10). However, *R. odorabile* retains carbon at an order of magnitude lower than most other marine sponges retaining carbon in the form of ultraplankton (ranging between 16.7 and 55.2 μg $C.l^{-1}$) (Table 5.10). This lower retention of carbon is directly attributed to their narrow diet across shelf locations compared to the broad diet identified for other marine sponges that generally retain large percentages of heterotrophic bacteria, *Prochlorococcus* spp., *Synechococcus* spp, picoeukaryotes <3 μm and nanoeukaryotes <10 μm (Pile et al. 1996; Pile 1997; Yahel et al. 2003; Pile and Young 2006).

Table 5.10 Summary of published literature detailing the mean retention of particulate organic carbon (POC) and dissolved organic carbon (DOC) by marine sponges. The ultraplankton cell types retained by each sponge species are indicated and the data for POC and DOC retained has been adjusted to a uniform measure (μg C l^{-1} filtered). (Bac – heterotrophic bacteria, Pro –

Prochlorococcus spp., Syn – *Synechococcus*-type cyanobacteria, Pico – picoeukaryotes <3 μm, Nano – nanoeukaryotes <10 μm, Protist – nano-planktonic protists, Unarm – unarmoured cells, Arm – armoured cells, Unresolved – unresolved particulate organic matter, n/a – not available).

Sponge species	POC (μg C.l^{-1} filtered)	DOC (μg C.l^{-1} filtered)	Ultraplankton consumed
Aphrocallistes vastus[1]	26.4 ± 15.6*	-	Bac, Protist
Rhabdocalyptus dawsoni[1]	22.8 ± 10.8*	-	Bac, Protist
Sericolophus hawaiicus[2]	34.1	-	Bac, Pro, Syn, Pico
Theonella swinhoei[3]	24.0 ± 12.0*	120.0 ± 84.0*	Bac, Pro, Syn, Pico, Nano
Ircinia felix[4]	7.0	-	Bac, Syn
Ircinia strobilina[4]	43.0	-	Bac, Pro, Syn, Nano
Mycale lingua[5]	55.2	-	Bac, Pro, Syn, Pico, Nano
Verongia fistularis[6]	16.7 ± 1.4*	-	n/a
Verongia gigantea[7]	20.1	-	Bac, Unarm, Arm, Unresolved
Mycale sp.[7]	26.1	-	Bac, Unarm, Arm, Unresolved
Tethya crypta[7]	39.7	-	Bac, Unarm, Arm, Unresolved
Rhopaloeides odorabile[8]	3.0 – 9.3[#]	-	Bac, Pico

[1] – Yahel et al. (2007); [2] – Pile and Young (2006); [3] – Yahel et al. (2003); [4] – Pile (1997); [5] – Pile et al. (1996); [6] – Reiswig (1981); [7] – Reiswig (1971a); [8] – This study; * – Mean ± SE; [#] – Range.

The exhalant current velocities of *R. odorabile* (ranging between 0.02 and 31.90 cm.s^{-1}) across shelf locations are within the range of exhalant current velocities measured for other shallow water demosponges previously published. Exhalant velocities for *R. odorabile* at Pelorus Island and Rib Reef are comparable to *Baikalospongia bacilifera* (3.3 cm.s^{-1}) (Savarese et al. 1997), *Halicona ureolus* and *Halicona panicea* (5.56 cm.s^{-1}) (Riisgard et al. 1993), *Theonella swinhoei* (7.6 cm.s^{1}) (Yahel et al. 2003), *Mycale* sp. (7.8 cm.s^{-1}) (Reiswig 1971b), and *Verongia fistularis* (10.16 cm.s^{-1}) (Reiswig 1981). Exhalant velocities for *R. odorabile* on Pith Reef are comparable to *Tethya crypta* (12.8 cm.s^{-1}) (Reiswig 1973), *Mycale lingua* (14.0 cm.s^{-1}) (Pile et al. 1996) and *Verongula* sp. (17.3 cm.s^{-1}) (Reiswig 1974). As sponge pumping activity is directly related to their metabolic costs (Riisgard et al. 1993), comparable levels of pumping activity between *R. odorabile* and other tropical and temperate sponges suggests that *R. odorabile* may need to retain similar high levels of carbon to other sponges to meet its nutritional requirements.

Given the low retention of ultraplankton, *R. odorabile* may obtain its nutritional requirements from other sources, in particular DOC. The retention of DOC has been identified as an important nutritional pathway for coral reef sponges possessing symbiotic microbial communities, contributing up to 90% of the total organic carbon retained (Reiswig 1990; Yahel et al. 2003; de Goeij et al. 2008). Three different coral reef sponges, *Halisarca caerulea*, *Mycale microsigmatosa*, and *Merlia normani*, all possessing sponge-associated bacteria (in excess of 1.0×10^9 bacteria.cm^{-3}.sponge^{-1}) retain DOC at two orders of magnitude higher than carbon obtained from the retention of bacteria (de Goeij et al. 2008). *R. odorabile* individuals also possess a diverse and abundant microbial community (approximately 1×10^{10} bacteria.cm^{-3}.sponge^{-1}) where bacteria are distributed not only throughout the mesohyl, but they also line pinacoderm tissues and surround the choanocyte chambers (Webster and Hill 2001; Webster et al. 2001a). Webster and Hill (2001) suggested that given the high abundance of bacteria and their distribution around choanocyte chambers, as well as lining pinacoderm tissues, these bacteria might play a central role in nutrient uptake in *R. odorabile*. However, additional studies are required to elucidate the uptake of DOC by *R. odorabile*, and if present, determine whether this large microbial community is responsible for the uptake of DOC.

In conclusion, *R. odorabile* is a poor consumer of ultraplankton, a primary food source for many benthic filter-feeding invertebrates. The selective retention of picoeukaryotes <3 μm between months and across broad spatial scales, demonstrates that natural environmental gradients do not influence the type, or efficiency, of ultraplankton retained by actively pumping individuals. Despite picoeukaryotes <3 μm (the main planktonic food source of *R. odorabile*) being more abundant on inner-shelf reefs than mid- and outer-shelf reefs, they were retained with high efficiencies regardless of shelf location. As a result 1.5 and 3 times more carbon and nitrogen (in the form of picoeukaryotes <3 μm) were retained on inner-shelf reefs than mid- and outer-shelf reefs, respectively. Paradoxically, this higher retention of carbon and nitrogen does not reflect the

abundance, size and reproductive output of *R. odorabile* across shelf locations (Chapter 2, Whalan et al. 2007a).

Given the differences in sediment grain size and mineralogy across shelf locations of the central GBR, I hypothesise that the smaller individuals, lower abundance and lower reproductive output of *R. odorabile* on inner-shelf reefs compared to outer-shelf reefs may be explained by *R. odorabile* being exposed to fine clay sediments on inner-shelf reefs that are absent from outer-shelf reefs (Chapter 4). These fine clay sediments may clog the filtering system of *R. odorabile* resulting in increased physiological stress and energy expenditure to remove these clay sediments. Therefore, Chapter 6 will elucidate the physiological response of *R. odorabile* to suspended sediments of similar grain size but of contrasting mineralogical composition.

Chapter 6 – Physiological Responses of *Rhopaloeides odorabile* to Suspended Sediments of Contrasting Mineralogical Compositions

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6.1 Introduction

This chapter builds on the findings of Chapter 4 which characterised the suspended sediment material within the habitat of *R. odorabile*. *R. odorabile* is exposed to distinct changes in the mean grain size and mineralogical composition of suspended sediments across shelf locations of the central GBR. Individuals on inner-shelf reefs are exposed to fine clay sediments (approximately 80% of suspended sediment and <10 μm) whereas those on mid- and outer-shelf reefs are exposed to coarse carbonate sediments (approximately 70% of suspended sediment are >100 μm).

It has been well established that in contrasting benthic habitats the structure, biomass and metabolism of marine benthic communities are primarily influenced by sedimentation (Balata et al. 2005). Increased sedimentation in tropical coastal reef habitats has resulted in declining coral cover,

reduced biodiversity, lower recruitment and a change in community structure (reviewed in Fabricius 2005). Similarly, increased sedimentation in temperate coastal reef habitats leads to a reduction in species richness, diversity and abundance of benthic organisms (Airoldi and Cinelli 1997; Connell 2005).

Increased exposure to sediments in coastal reefs negatively impacts the physiology of coral taxa, leading to reductions in photosynthetic efficiency and increased relative respiration resulting in bleaching, reduced reproductive output and tissue necrosis (reviewed in Fabricius 2005). However, more recently these negative impacts have been specifically linked to the contrasting physical, organic and mineralogical properties of different sediments (Weber et al. 2006). Despite the plethora of literature detailing the impacts of sediments on coral physiology and ecology there are few studies examining the physiological impacts of sediments and sedimentation on coral reef sponges. This is surprising given sponges form an integral component of coral reef systems (Wilkinson and Cheshire 1989), not only providing structural rigour to coral reef frameworks (Wulff and Buss 1979; Wulff 1984, 2001), but also playing a pivotal role in energy transfer processes (Pile 1997) and in providing refuge for other coral reef organisms (reviewed in Wulff 2006).

For sponges, sedimentation influences the structure, abundance and diversity of sponge assemblages in temperate and tropical reef habitats (Zea 1994; Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006). In addition, high concentrations of fine suspended sediments clog inhalant canals and the filtering apparatus of sponges, resulting in reduced pumping activity of sponges (Gerrodette and Flechsig 1979) suggested to influence both feeding efficiencies and respiration of sponges (Reiswig 1971b; Gerrodette and Flechsig 1979). Energy loss associated with clogged inhalant canals and filtering structures has been further suggested to be responsible for reduced growth rates (Roberts et al. 2006) and low reproduction of sponges in high sediment environments (Roberts et al. 2006; Whalan et al. 2007a). Despite these assumptions there have been no studies that have

characterised energy losses associated with clogged inhalant canals and filtering structures of sponges, nor reports that have elucidated the impact of suspended sediments on the energy budgets of sponges. More importantly, it is unclear what impact suspended sediments have on the basic metabolic response of sponges, and whether this response changes with exposure to suspended sediments with distinctly different mineralogical compositions.

R. odorabile has a broad distribution and is found across inner-, mid- and outer-shelf reef environments of the central GBR. On inner-shelf reefs where suspended sediments are dominated by fine terrestrial sediments, *R. odorabile* has a significantly lower abundance (Chapter 2) and lower reproductive output (Whalan et al. 2007a) compared to mid- and outer-shelf reefs, where these fine terrestrial sediments are absent. Coarse carbonate sediments dominate mid- and outer-shelf reefs where *R. odorabile* has a consistently higher abundance (Chapter 2) and a much higher reproductive output than inner-shelf reef populations (Whalan et al. 2007a), indicating a more optimal offshore environment. However, the ability of *R. odorabile* to live in these contrasting environments with distinctly different compositions of suspended sediments provides us with an excellent model species to investigate the impacts of sediment and sedimentation on sponge physiology and the resultant outcomes in terms of fitness (growth and reproduction). This chapter investigates the effect of suspended sediments with contrasting mineralogical compositions on the metabolic response of *R. odorabile*. More specifically, *R. odorabile* is used to (1) investigate differences in the respiration response of sponges with short-term exposure to fine suspended clay and carbonate sediments, and (2) investigate the impacts of longer-term exposure of clay sediments on the respiration rate of sponges.

6.2 Materials and methods

6.2.1 Collection of specimens

During November 2006, 50 sponge explants were cut from 50 *R. odorabile* (1 explant per individual) at Rib Reef (18°29.97'S, 146°52.52'E) on the central GBR. All sponge explants were placed into two plastic moulded recovery cages (Aqua-Tech) that were anchored to the sea floor at 9 m (the same depth that the sponges were collected from). Explants were left in these cages *in situ* for nine months to ensure that all cut surfaces had healed (Louden et al. 2007).

Sponge explants were collected from Rib Reef during July and September 2007 and transported to the aquarium facilities at James Cook University (JCU) on the same day as collection. Sponges were placed into a large flow through oval tank (800 l) and acclimatised to laboratory conditions for 3 d prior to conducting respirometry experiments. The aquarium facilities were temperature controlled for the entire experimental period. Water temperatures were maintained at 23°C and 25°C during July and September, respectively. These temperatures reflected ambient temperatures on the reefs where the sponge explants were collected. To provide adequate water flow around each sponge explant they were placed onto mesh racks that were mounted 5 cm above the bottom of the tank. Circulation was maintained within the oval tank by four power head pumps (Aqua-clear 702). Seawater within the aquarium system was collected from Cape Cleveland 50 km south of Townsville, and was filtered to 10 μm. Bio-filtration, foam fractionators and sand filters were used to maintain water quality. Each aquarium was illuminated on a 10:14 h light:dark regime. Prior to running experiments surface-associated fouling organisms were carefully removed from the surfaces of all individuals to ensure that the respiration rates measured were only those of the sponge and not other organisms attached to their surfaces.

6.2.2 Respirometry design

To determine the respiration rate of *R. odorabile* in response to contrasting mineralogical inorganic suspended sediments, respirometry experiments were carried out using recirculating transparent perspex flow chambers (2.7 l) fitted with calibrated Clark-type oxygen electrodes (Cheshire systems, Adelaide). The general set-up of this respirometry system is described in detail in Chapter 3 (Section 3.2.2), however, slight modifications were made to this system for both the short and long-term exposure experiments as described below.

6.2.3 Short-term exposure experiment

The metabolic response of *R. odorabile* to suspended sediments of different mineralogical compositions was quantified in July 2007. The respiration rates of 30 *R. odorabile* were measured for a 7 h period whilst exposed to both clay and carbonate suspended sediments (n=5) of similar size (see below) and a controlled treatment (1 μm filtered seawater, n=5 per treatment). A kaolinite clay standard (KGA-1B, Source Clays Repository Purdue University, Indiana) with a mean grain size of 3.1 ± 0.1 μm was used for the clay sediment treatment, while the carbonate sediment treatment consisted of crushed dead coral and *Halimeda* spp. skeletons with a mean grain size of 8.2 ± 0.7 μm. The slight differences in mean grain size of the two sediment types may influence the result, however, it was not possible to crush the coral and *Halimeda* spp. carbonate skeletons to exactly the same size as clay sediments. Therefore caution is taken when interpreting the results.

Respiration rates were measured in four closed recirculating transparent Perspex flow chambers (as described in Chapter 3, Section 3.2.2). Each flow chamber was maintained in individual 80 l circular tanks to enable the respiration rates of *R. odorabile* exposed to clay, carbonate and control treatments to be measured simultaneously. Each tank was fitted with two power head pumps

(Aquaclear 702) to create a homogeneous environment with sufficient circulation to maintain the sediments in suspension.

Respiration rates were measured consecutively over a 5 d period. During each day of data collection 6 explants were randomly placed into three of the four respiration chambers (2 sponges per chamber). Two of the four chambers were subjected to the sediment treatment (one clay and one carbonate) at an initial concentration of 64 $mg.l^{-1}$. However, due to the settlement of sediments on the experimental chambers, sponge surfaces and on pumps and tanks, experimental sponges were exposed to suspended sediment concentrations ranging between 34.5 ±5.2 and 64.8 ± 4.9 $mg.l^{-1}$ for clay sediments and between 34.1 ± 0.7 and 57.0 ± 2.2 $mg.l^{-1}$ for carbonate sediments. The exposure of sponges to these suspended sediment concentrations are ecologically relevant to suspended sediment concentrations <100 $mg.l^{-1}$ in coastal coral reefs environments of the central GBR, with some fringing reefs also experiencing suspended sediment concentrations up to 200 $mg.l^{-1}$ under extreme conditions (Larcombe et al. 1995). The third chamber was a control chamber with a sponge but no sediment, and the fourth chamber remained empty during the respiration run to control for photosynthesis and respiration of micro-organisms within the water. Chambers were cleaned at the end of each run to prevent bio-film formation (Hoogenboom et al. 2006) and to remove accumulated sediments. To obtain a baseline rate (time 0) respiration was measured for 2 h at the start of the experiment prior to sediments being added to each of the treatments. Respiration rates were measured for the 7 h period after the sediment was added for each treatment. All sponges were visually assessed for open oscula immediately following each daily experiment, and pumping activity was visualised with fluorescein dye (Pile et al.1996). After this visual confirmation, whole explants were weighed to obtain wet weights, allowing respiration rate to be compared to literature values ($\mu mol\ O_2\ g.fw^{-1}.h^{-1}$).

6.2.4 Long-term exposure and recovery experiment

Due to the higher overall respiration response of *R. odorabile* to short-term exposure of suspended clay sediments compared to carbonate sediments (see results) and the exclusive abundance of fine clay sediments on inner-shelf reef locations (Chapter 4), the effects of long-term exposure of suspended sediments on the respiration rate of *R. odorabile* were examined using fine clay sediments.

To quantify the metabolic response of *R. odorabile* to long-term suspended sediment exposure 20 *R. odorabile* explants were placed into 10 separate 20 l glass aquaria (two explants per aquarium). Five of these aquaria were exposed to suspended clay sediment for 4 d after which the sponges were then allowed to recover for a further 4 d in the absence of sediment. The remaining five aquaria were maintained as control aquaria for the duration of the experiment (1 μm filtered seawater). Each aquarium had a mesh rack to keep sponges in mid water and a power-head pump (Aquaclear 702) to provide current flow in the tank to maintain sediments in suspension. The clay sediments added to the aquaria had a mean grain size of 3.1± 0.1 μm and were added to the aquaria at a concentration of 64 mg l^{-1}. However, due to the settlement of sediments, suspended sediment concentrations ranged between 29.8 ± 3.0 and 64.7 ± 1.4 mg l^{-1} over consecutive 12 h periods. After each 12 h period each aquarium was cleaned and the sediment replaced to maintain suspended sediment concentrations throughout the duration of the experiment.

Respiration rates of *R. odorabile* were measured using three closed recirculating transparent flow chambers maintained in separate 80 l circular tanks (as described above in section 6.2.3) over the 8 d experimental period. During each day of data collection respiration rates were measured randomly for both of the sponges in each of the control and treatment aquaria for a 1 h period. This resulted in consecutive respiration measurements for all 10 aquaria over a 5 h period. During each of the five 1 h measurement periods both paired sponges within each of the treatment and control aquaria were

placed into two of the three respirometry chambers (two sponges from a single treatment per chamber). The third chamber remained empty during the respiration run to control for photosynthesis and respiration of micro-organisms within the water. At the end of each run, sponges were removed from the chambers and placed back into their respective experimental aquaria. Chambers were cleaned daily to prevent bio-film formation (Hoogenboom et al. 2006). To obtain a baseline rate (time 0) respiration was measured for each pair of sponges for 1 h prior to sediments being added. After the addition of sediment, respiration rate was measured for both treatment and control sponges daily for the following 4 d exposure period. Respiration rates were further measured during the recovery period at day one (day one of recovery), day two (day two of recovery) and day four (day four of recovery). Respiration rates were not measured during the day three recovery period as the computer malfunctioned. During each day of the experiment all experimental sponges were visually assessed for open oscula, and their pumping activity was visually confirmed with fluorescein dye (see previous detail). Immediately following the longer-term exposure experiment whole explants were weighed to obtain wet weights, allowing respiration rate to be compared to literature values (μmol O_2 $g.fw^{-1}.h^{-1}$).

6.2.5 Statistical analysis

To determine if the respiration rate of *R. odorabile* differed in response to short-term exposure of suspended sediments of contrasting mineralogical compositions (clay and carbonate sediments) the respiration data for *R. odorabile* was analysed with a univariate repeated measures ANOVA model. The respiration rate measured at time 0, 1, 2, 4 and 7 h intervals comprised the within factor treatment, and the two different suspended sediment mineral compositions (clay and carbonate) including the no sediment control comprised the between factor treatment.

To determine if the respiration rate of *R. odorabile* differs in response to long-term exposure to suspended fine clay sediments over a 4 day exposure / 4 day recovery period, the respiration data for *R. odorabile* was analysed with a univariate repeated measures ANOVA model. The respiration rate measured at exposure day 1, 2, 3 and 4 and recovery day 1, 2 and 4 (i.e. time) comprised the within factor treatment and the suspended clay sediment and no sediment control comprised the between factor treatment.

To ensure all data met the assumptions of the statistical analyses performed all data were checked for homogeneity of variance and normality using standardised residuals versus predicted value plots and Q-Q plots of residuals, respectively. In addition, statistical significance for both repeated measures ANOVA models were estimated from the Greenhouse-Geisser adjusted probability to avoid violating the assumption of circularity (sphericity) of the within-subject (time) variance-covariance matrix (von Ende 1993).

6.3 Results

6.3.1 Short-term exposure experiment

All sponges were visually confirmed to have multiple oscula open prior to exposing the sponges to the suspended sediments,. All sponges were actively pumping prior to the addition of suspended sediments as determined by the uptake and release of fluorescein dye by sponges.

The rates of respiration for *R. odorabile* were similar between clay, carbonate and the control treatment explants at time 0, with individuals having mean respiration rates of 1.19 ± 0.17, 1.08 ± 0.19 and 1.03 ± 0.20 μmol O_2 $g.fw^{-1}.h^{-1}$, respectively (Figure 6.1). Mean respiration rates did not differ significantly from the control treatments After being exposed to fine clay and carbonate sediments for 7 h, (Figure 6.1, Table 6.1). However, the mean respiration rate of *R. odorabile* exposed to fine clay sediments increased by 35% from the baseline rate of respiration after 7 h (1.60

± 0.33 μmol O_2 g.fw^{-1}.h^{-1}), while individuals exposed to carbonate sediments increased their mean respiration rate by only 12% (1.22 μmol O_2 g.fw^{-1}.h^{-1}). The mean respiration rate of the control sponges remained the same throughout the experiment. Visual observations of treatment and control sponges post exposure confirmed that multiple oscula were open for each sponge. In addition all sponges were pumping post experimental manipulation as determined by the visual uptake and release of fluorescein dye from the surrounding water. Furthermore, all surfaces of the sponges exposed to both the fine clay and the fine carbonate sediments were heavily covered in sediment, with visual evidence of mucus production by sponges exposed to the clay treatment after 7 h of exposure.

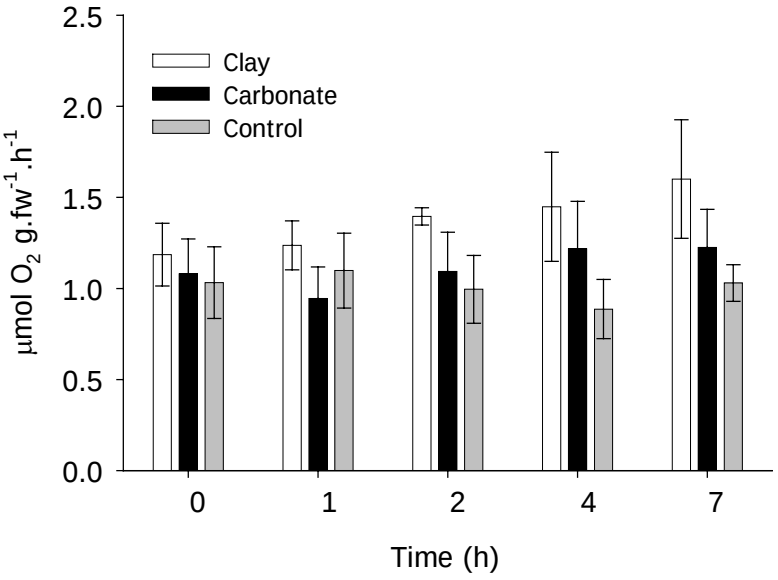

Figure 6.1 Mean respiration rate (μmol O_2 g.fw^{-1}.h^{-1}, ± SE, n=5) of *R. odorabile* explants exposed to suspended clay and carbonate sediments over a 7 h period. Time 0 represents the baseline respiration rate of *R. odorabile* before the addition of sediments, while time 1, 2, 4 and 7 represent the hours post sediment addition.

Table 6.1 Repeated measures ANOVA model comparing the mean respiration rate of *R. odorabile* in the presence of suspended clay and carbonate sediments at ecologically relevant concentrations over a short-time period. Significance values are based on the Greenhouse-Geisser corrections.

Source	df	MS	F	*P*
Within subjects				
Time	1.603	0.227	0.95	0.386
Time x treatment	3.206	0.181	0.76	0.539
Error	19.233	0.235		
Between subjects				
Treatment	2	0.885	1.36	0.295
Error	12	0.653		

6.3.2 Long-term exposure and recovery experiment

Once again, all sponges were visually confirmed to have multiple open oscula and were actively pumping prior to exposing sponges to fine clay sediments,.

The baseline respiration rate (Time 0) between control and treatment individuals were similar with respiration rates of 1.75 ± 0.12 and 1.46 ± 0.19 μmol O_2 $g.fw^{-1}.h^{-1}$, respectively (Figure 6.2). Over the 8 d experimental period there was a significant interaction between treatment and time (P = 0.043) demonstrating that the respiration rate of *R. odorabile* was influenced both over time and between treatments (Table 6.2, Figure 6.2). However, the highly significant between-subject effect of treatment (Table 6.2) indicates that the significant interaction term was driven primarily by differences in the respiration rate between treatment and control sponges over time. After 24 h of exposure to fine clay sediments the respiration rate of treatment sponges (2.51 ± 0.14 μmol O_2 $g.fw^{-1}.h^{-1}$) increased by 43% of their baseline respiration rate, and were almost double the rate of respiration of the control sponges (1.28 ± 0.15 μmol O_2 $g.fw^{-1}.h^{-1}$). These respiration rates remained consistent for up to 2 d of exposure to fine clay sediments. After 2 d of exposure respiration rates began to decrease, with respiration rates of treatment sponges (2.08 ± 0.16 μmol O_2 $g.fw^{-1}.h^{-1}$)

declining to only 19% higher than their baseline rate of respiration after 4 d of exposure. However, the respiration rates of treatment sponges were still 1.5 times greater than the respiration rates of the control sponges. After day 4 the sediment exposure was stopped and both the treatment and control sponges were allowed to recover for 4 d. After the first day of recovery respiration rates of treatment sponges (1.65 ± 0.20 μmol O_2 $g.fw^{-1}.h^{-1}$) declined and were similar to their baseline respiration rate, although they were about 1.7 times greater than the respiration rates of the control sponges (0.96 ± 0.08 μmol O_2 $g.fw^{-1}.h^{-1}$). However, after 4 d of recovery the respiration rates of the treatment sponges (1.66 ± 0.16 μmol O_2 $g.fw^{-1}.h^{-1}$) remained similar to baseline respiration rates and were also similar to the respiration rate of the control sponges (1.35 ± 0.17 μmol O_2 $g.fw^{-1}.h^{-1}$) (Figure 6.2).

During the first and second day of sediment exposure oscula of both treatment and control sponges were open and actively pumping. In addition, treatment sponges were producing a mucus sheet to remove clay sediments from their surfaces. However, after the first two days until after the first day of recovery oscula of the treatment sponges started to shrink in size, while some completely closed. Pumping activity was still evident for both treatment and control sponges during this period, and treatment sponges were still producing a mucus sheet that was readily flaking off. After day four of recovery oscula of treatment sponges were still shrunk and pumping activity was still evident in control and treatment sponges. In addition, mucus sheets were still readily flaking off the treatment sponge surfaces while some initial signs of tissue necrosis for treatment sponges were evident.

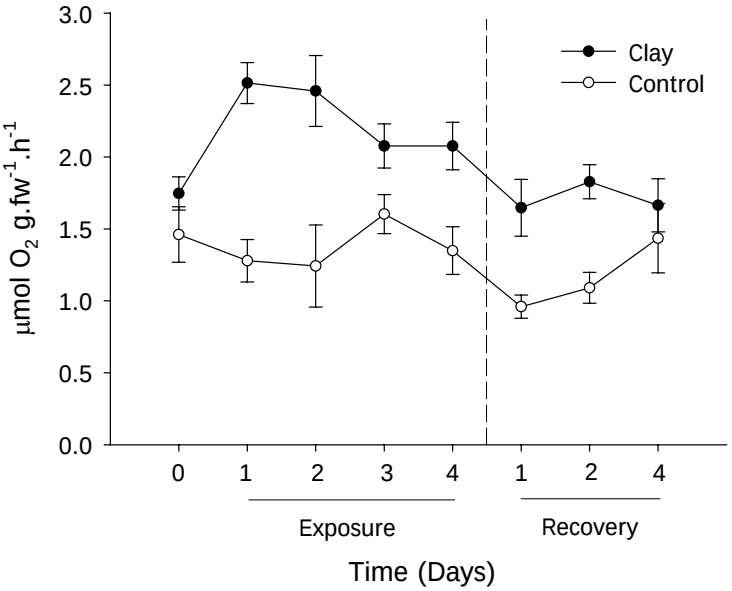

Figure 6.2 Mean respiration rate (µmol O_2 g.fw^{-1}.h, ± SE, n=5) of *R. odorabile* over a 4 d period exposed to suspended clay sediments, and their subsequent recovery over a 4 d period without sediment exposure. Time 0 represents the baseline respiration rate of *R. odorabile* before the addition of sediments, while time 1, 2, 3, and 4 (exposure) represent the 4 d during sediment exposure. Time 1, 2, 4 (recovery) represent the 4 d recovery period without sediment addition.

Table 6.2 Repeated measures ANOVA model comparing the respiration rate of *R. odorabile* in the presence of suspended clay sediments and control sponges at ecologically relevant concentrations over a long-time period. Significance values are based on the Greenhouse-Geisser corrections.

Source	df	MS	F	*P*
Within subjects				
Time	3.354	0.923	3.71	0.020
Time x Treatment	3.354	0.746	2.99	0.043
Error	26.834	0.249		
Between subjects				
Treatment	1	9.769	38.95	<0.001
Error	8	0.251		

6.4 Discussion

This study identified, for the first time, that suspended sediments with distinctly different mineralogical compositions but of similar size and at ecologically relevant concentrations induce different respiration responses in a tropical sponge. Short-term exposure (7 h) of *R. odorabile*

individuals to fine carbonate sediments induced only a slight increase in respiration rate (12%) from their baseline respiration rate, while short-term exposure to fine clay sediments rapidly increased the respiration rate of *R. odorabile* (35%) from their baseline respiration rate. This elevated respiration response may be a combination of the observed mucus production on the exposed surfaces of *R. odorabile* and continual pumping activity. Interestingly, when exposed to these fine clay sediments for extended periods (up to 4 d) the respiration response of *R. odorabile* remained elevated (up to 43%) from their baseline respiration rate. This is despite observations of physical shut down, where oscula begin to close and pumping rates appear to slow down. The continual production of mucus to remove fine clay sediments from their exterior surfaces may contribute to this elevated respiration rate over time. At the conclusion of the experiment tissue necrosis was evident for sponges exposed to fine clay suspended sediments.

The elevated respiration rate of *R. odorabile* exposed to fine clay sediments compared to fine carbonate sediments may be a direct function of the different properties of the suspended sediments. Clay sediments have a high specific surface area, and their surfaces are electrically charged (Hunter and Liss 1979). As a consequence clay particles have adhesive surface properties and can behave very “sticky” (Wang and Lee 1993). In contrast, carbonate sediments have a low specific surface area and their surfaces are inert (non “sticky”) (Milliman 1974). The adhesive property of clay particles facilitates the smothering of sponges, including exterior surfaces and the internal surfaces of inhalant canals. In addition, when sponges filter these clay sediments they may also clog the fine filtering apparatus of sponges affecting pumping and feeding (Reiswig 1971b; Gerrodette and Flechsig 1979). For example, Gerrodette and Flechsig (1979) identified a 40% reduction in pumping activity for the tropical sponge *Verongia lacunose* when exposed to fine clay suspended sediments. Therefore individuals exposed to fine clay particles may require additional energy efforts to remove these sediments from their surfaces and to unclog inhalant canals and/or filtering apparatus. In contrast, the non-adhesive properties of carbonate sediments suggests that they do not

smother sponges or impact on the efficiency of inhalant canals and filtering apparatus. Subsequently, sponges may require less energy to dislodge these carbonate particles from their surfaces, inhalant canals and filtering apparatus.

The impact of suspended sediments on the physiology of sponges is limited to only two studies. Despite both studies identifying the negative impacts of suspended sediments on the pumping activity of sponges (Reiswig 1971b; Gerrodette and Flechsig 1979) neither of these studies measured the metabolic costs associated with these impacts. However, both hypothesised that reduced pumping activity of sponges due to increased sedimentation may also result in significant reductions in respiration and feeding efficiencies (Reiswig 1971b; Gerrodette and Flechsig 1979). The results from the present study contradict these earlier hypotheses, unequivocally demonstrating that sponge respiration rapidly increases in response to suspended fine clay sediments at ecologically relevant concentrations and sizes. In addition, the visual observations made during these experiments suggest that mucus production may be responsible for the elevated and sustained respiration response of *R. odorabile.* Furthermore, evidence of tissue necrosis for *R. odorabile* after longer-term exposure to clay sediments suggests that *R. odorabile* may invest considerable amounts of energy to remove fine clay sediments from their surfaces and filtering apparatus.

Whilst marine sponges initiate the production of mucus for defence against sedimentation (Turon et al. 1999) it is unclear what metabolic costs are associated with producing a mucus sheath. This is despite earlier studies identifying the impact of mucus production on coral energetics (Benson and Muscatine 1974; Richman et al. 1975). The daily production of mucus by corals is metabolically very draining and can cost corals up to 2.5 times the amount of carbon they produce in 24 h (Riegl and Branch 1995). Although the cost of mucus production by sponges is unclear, the fact that *R. odorabile* increased its respiration rate by almost 50% when exposed to fine clay suspended

sediments, and the initial signs of tissue necrosis indicate that the production of mucus by sponges may be very costly. Sponges may use other physiological responses in addition to mucus production to deal with excessive sediments that may also be energetically costly. For example, sponges are capable of water flow reversal, a potential mechanism to back-flush their system of canals and feeding chambers to remove excessive sediments (Simpson 1984). Sponges may also reorganise their internal anatomy by continuous cell movements, enabling new ostia and oscula to be opened to help overcome clogging (Bond 1992; Ilan and Abelson 1995). Furthermore, sponges are also capable of gross morphological modification to allow for adaptation to the changing environment (Bell et al. 2002), whilst some sponges actively crawl away from sites of high sediment exposure (Maldonado and Uriz 1999).

While the results from this study clearly demonstrate an increase in respiration rate of *R. odorabile* in response to fine clays suspended sediments, these measurements may be conservative given laboratory conditions. Respiration rate of *R. odorabile* in response to suspended sediments may be higher in the field due to the contamination of sediments in inshore coastal habitats of the central GBR with heavy metals, pesticide residues and other pollutants (Müller et al. 1999; Haynes et al. 2000). The effects of heavy metals, pesticides and pollutants can have negative effects on the health and fitness of sponges (Cebrian et al. 2003, 2006; Cebrian and Uriz 2007a,b). Exposures to heavy metals, hydrocarbons and other pollutants result in reduced pumping capacity of adult sponges (Cebrian et al. 2006), as well as causing irreparable DNA damage (Müller and Müller 1998; Schröder et al. 2006), leading to impaired cellular functions (Cebrian and Uriz 2007a), low reproductive output (Cebrian et al. 2003) and lower larval settlement (Cebrian and Uriz 2007a,b).

In conclusion, the results from this study provide an understanding of the respiration activity of coral reef sponges with massive morphologies that are exposed to fine suspended sediments with contrasting mineralogical compositions. The mineralogical composition of suspended sediments

were critical at triggering quite different respiratory responses by *R. odorabile*, this could purely be a physical reaction to surfactant properties of clays. However, it hints at the ability of sponges to recognise particle character and respond accordingly, which has previously been demonstrated for the sponge *Chondrosia reniformis* (Bavestrello et al. 2003). Earlier hypotheses suggest that sponges living in coastal environments exposed to heightened levels of sedimentation may experience periods of physiological shutdown and reduced rates of respiration (Reiswig 1971b; Gerrodette and Flechsig 1979). The findings presented here suggest otherwise, with sponges exposed to fine clay sediments significantly increasing their respiration rate while shutting down oscula and pumping activity. These results provide initial evidence to support the theory that sponges living in coastal environments subjected to high concentrations of terrigenous sediments are subjected to increased metabolic stress that may limit their growth and survival, reproductive output, recruitment and subsequently their distribution and abundance.

Chapter 7 – Synthesis and Discussion

There is a growing body of literature highlighting that the population dynamics of marine sponges in shallow water coastal reef habitats are influenced by environmental factors, including sediment (Zea 1994; Bell and Smith 2004; Carballo 2006; Maldonado et al. 2008), light (Wilkinson and Trott 1985; Jokiel 1980), food availability (Storr 1976; Wilkinson and Cheshire 1989; Tanaka 2002; Lesser 2006), water flow and exposure (Wilkinson and Evans 1989; Bannister et al. 2007) and pollution (Cebrian and Uriz 2007a,b). Of these environmental factors, sedimentation, light, and food availability have been proposed as the key to structuring population dynamics of sponges along natural environmental gradients on cross-shelf reef systems (Wilkinson and Cheshire 1989; Wilkinson and Evans 1989; de Voogd et al. 1999; Cleary et al. 2005). Increased anthropogenic inputs into coastal reef systems including elevated levels of sedimentation, nutrients and pollutants

associated with increased terrigenous runoff, have exacerbated these natural environmental gradients (Neil et al. 2002; McCulloch et al. 2003; Devlin and Brodie 2005). However, it is unclear how these changes impact on sponge population dynamics. Developing an understanding of the role of key environmental factors associated with these environmental gradients in structuring the dynamics of individual sponge species is necessary to develop key management strategies to minimise and quantify anthropogenic changes on the marine environment. Using *Rhopaloeides odorabile* as a model, the results of this study contribute to an improved understanding of the role of environmental gradients in structuring the dynamics of sponges on cross-shelf reef systems.

The major findings of this study are:

1. Chapter 2: *R. odorabile* is three times more abundant on mid- and outer-shelf reefs than inner-shelf reefs. Individuals on mid- and outer-shelf reefs are more abundant at a depth of 10 m than individuals on inner-shelf reefs preferring shallower depth distributions. Abundance and depth distributions may be related to light, food availability and suspended sediments.
2. Chapter 3: Light is not a factor in explaining abundance, size and depth distribution patterns of *R. odorabile* between reefs sampled across shelf locations due to the absence of photosymbionts and the subsequent lack of photosynthesis.
3. Chapter 4: There are distinct differences in grain size and mineralogy of suspended sediments between inner- and outer-shelf reef habitats. The dominance of fine clay suspended sediments on inner-shelf reefs and their absence on outer-shelf reefs suggests they restrict the abundance and depth distribution of *R. odorabile* on inner-shelf reefs. This provides a testable experimental hypothesis for this environmental factor (Chapter 6).
4. Chapter 5: There is increased food availability on inner-shelf reefs including picoeukaryotes <3 μm which are the main planktonic food source for *R. odorabile* (with the exception of heterotrophic bacteria during February). Picoeukaryotes <3 μm are retained preferentially

with high efficiencies and these efficiencies are consistent across shelf locations. Given the higher abundance of picoeukaryotes <3 μm on inner-shelf reefs, *R. odorabile* assimilated three times more carbon and nitrogen on inner-shelf reefs than on outer-shelf reefs. Paradoxically, *R. odorabile* retains more food, and subsequently more carbon and nitrogen on inner-shelf reefs, however *R. odorabile* on inner-shelf reefs is not larger or more abundance than on outer-shelf reefs (Chapter 2). This may be explained by increased energetic costs for *R. odorabile* living in high sediment inner-shelf reefs exposed to fine clay suspended sediments (Chapters 4 and 6).

5. Chapter 6: *R. odorabile* exposed to fine clay sediments (as a proxy for inshore environments, Chapter 4) increased their respiration rate by 35% from their baseline rate of respiration. This compares to individuals exposed to carbonate sediment (as a proxy for offshore environments, Chapter 4) with a 12% increase from their baseline rate of respiration. Long-term exposure to clay sediments resulted in an even higher (43%) increase in respiration from their baseline rate of respiration, and this rate was double that of control sponges.

In conclusion, light availability is not a key factor explaining the differences in the abundance, size and distribution of *R. odorabile* between reefs across shelf locations of the central GBR. In addition, food availability and the retention of carbon and nitrogen by *R. odorabile* is counter intuitive, with increased abundance, size and reproduction of *R. odorabile* on outer-shelf reefs, demonstrating that food availability is not a key factor explaining differences in abundance, size and distribution of *R. odorabile* across shelf locations. However, distinct differences in sediment grain size and mineralogy between inner-shelf reef locations and mid- and outer-shelf reef locations, and the increased physiological stress associated with inner-shelf reef sediments, suggest strongly that suspended sediments play the key role in structuring the abundance, size and reproduction of *R. odorabile* on cross shelf coral reef systems.

Sedimentation is a key factor in structuring sponge communities across a broad range of temperate and tropical benthic habitats (Zea 1994; Bell and Barnes 2000a,b; Bell and Smith 2004; Carballo 2006). The results obtained during this study support this paradigm, but highlight that the mechanisms by which sediments affect the abundance, size and distribution patterns of sponges, are more complex than first thought. The results presented within this thesis demonstrate unequivocally that both grain size and mineralogy of suspended sediments are important factors affecting the physiology, ecology and reproductive biology of *R. odorabile* across the continental shelf of the central GBR. In a similar manner, a recent study by Maldonado et al. (2008) correlated increased sponge mortality with contrasting sediment grain sizes. This, coupled with the results obtained within this thesis, highlights the importance of understanding suspended sediment grain size and mineralogy in addition to sediment deposition rates when assessing the population dynamics of sponges. This study demonstrated, for the first time, elevated respiration rates of a tropical sponge exposed to fine clay suspended sediments. Elucidating the physiological niche boundaries (respiration, reproduction and growth) of *R. odorabile* to both clay and carbonate sediments at a range of ecologically relevant concentrations and grain sizes will provide an opportunity to develop models to assess the impacts of anthropogenic change on sponge physiology, reproduction and subsequently the abundance and distribution of sponges.

Light availability is a key factor influencing the depth distribution of phototrophic sponges across shelf locations on coral reef systems (Wilkinson and Trott 1985; Wilkinson and Cheshire 1989; Cheshire and Wilkinson 1991). The absence of photosynthetic activity and photosymbionts of *R. odorabile* (Chapter 3) clearly demonstrates that the regulation of *R. odorabile* at these distinct depths across shelf locations is not driven by the requirement of light for energy acquisition by photosynthesis. However, distinct depth distributions of *R. odorabile* across shelf locations may be regulated by light availability and larval settlement behaviours, where *R. odorabile* larvae display

positive phototactic behaviour prior to settlement, coinciding with a preference to settle on light exposed surfaces (Whalan et al. in press). This preference for light exposed surfaces may be driven by the requirement of light to activate the biosynthesis of the secondary metabolites diterpene furanodials and their derivatives within the surface tissues of *R. odorabile* (Thompson et al. 1987). These compounds have been suggested as a deterrent against predators and fouling organisms (Thompson et al. 1987).

Food availability is a key factor influencing the structure and dynamics of sessile benthic filter feeding invertebrates (Gili and Coma 1998; Coma et al. 2000; Coma and Ribes 2003; Lesser 2006), where increased food availability influences the distribution and abundance patterns of sponges (Wilkinson and Cheshire 1989), whilst promoting increased retention efficiencies (Pile et al. 1997; Bell et al. 1999; Pile 2005) and growth rates of sponges (Lesser 2006; Trussell et al. 2006). However, the results presented in this thesis, coupled with the reproductive output of *R. odorabile* elucidated by Whalan et al. (2007a), are counter intuitive. The abundance, size and reproductive output of *R. odorabile* is greater on outer-shelf reefs, where food availability (picoeukaryotes <3 μm) and the assimilation of carbon and nitrogen are significantly lower compared to inner-shelf reefs. Low reproductive output, smaller sizes and lower abundance of *R. odorabile* on inner-shelf reefs may be explained by increased energy losses associated with the initiation of physiological mechanisms to prevent clogging of inhalant canals and filtering apparatus by fine clay sediments which are absent on outer shelf reefs (Chapter 6). Alternatively, a large proportion of suspended sediments on inner-shelf reefs are less than 10 μm (Chapter 4), which are within the same size range that *R. odorabile* and other sponges retain with high efficiencies (Chapter 5; Pile et al. 1996, Pile 2005). Sponges are non-selective filter feeders (Bergquist 1978) and do not possess a discrete mechanism to deal with turbid waters, unlike bivalves which produce pseudofaeces (Yahel et al. 2003). Therefore, it is plausible that *R. odorabile* individuals living in inshore coastal reef habitats may also be ingesting a large proportion of these smaller particles that have no nutritional value. As

a result, *R. odorabile* may spend additional energy trying to digest and metabolise these suspended particles without receiving any additional energy. This suggests that suspended fine clay sediments < 10 μm may be diluting the nutritional value of the water that *R. odorabile* individuals are actively filtering. The higher energetic drains associated with fine clay sediments may result in energy normally utilised for growth and reproduction being diverted to maintenance and survival, resulting in lower reproductive output, smaller individuals and subsequently lower sponge abundance. Understanding the underlying physiological mechanisms initiated during sediment stress and their impacts on the energy requirements for growth, reproduction and daily metabolic requirements is necessary to address the impacts of anthropogenic change on the ecology of sponges.

Finally, the results presented in this thesis demonstrate the importance of understanding key factors associated with environmental gradients when assessing the population dynamics of sponges on cross shelf reef systems. Clearly sedimentation, including sediment grain size and mineralogy, is a key driving factor impacting on the distribution, abundance and size of sponges in hard bottom coral reef habitats. However, it is impossible to conclude that sediment is the sole contributor to variations in the structure and dynamics of sponges given the high biomass and abundance of sponge species in traditionally high sediment environments (Wilkinson and Cheshire 1989; Bannister et al. 2007). Other biotic and abiotic factors may also be responsible in structuring sponge populations. These include, but are not limited to, larval recruitment and survival (Zea 1993; Maldonado and Young 1996, 1998), substrate type and angle (Bell and Barnes 2000a,b), wave and current exposure (Wilkinson and Evans 1989; Bannister et al. 2007), predation and competition (reviewed in Wulff 2006) and pollution (Cebrian and Uriz 2007a,b). Understanding the role of these biotic and abiotic factors was beyond the scope of this thesis. However, the results from this work ultimately provide pivotal information identifying the key environmental parameters linked with anthropogenic change on the dynamics and structure of an important component of the benthic community.

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Appendix 1

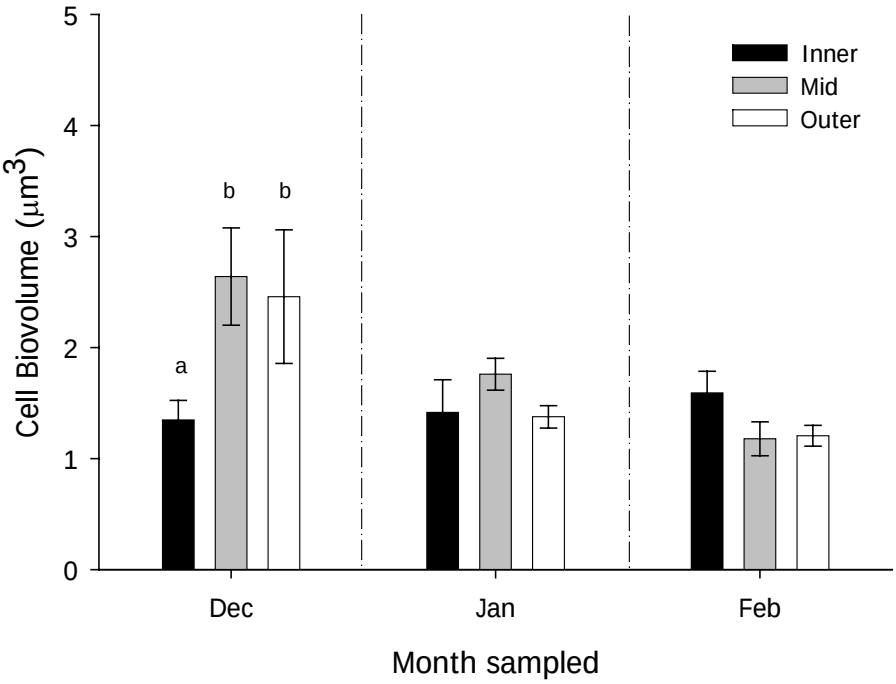

Figure A1 Mean cell biovolumes (μm^3, ± S.E.) of picoeukaryotes <3 μm across shelf locations and between months sampled. Superscripts denote significant differences (Tukey's HSD multiple comparison test, P <0.05 for log transformed data).

Table A1 Summary of a two-factor ANOVA comparing the mean bio-volumes of log transformed picoeukaryotes <3 μm across shelf locations and between months sampled. (Month: December, January, February; Shelf: inner-, mid-, and outer-shelf locations).

Source	df	M	F	*P*
Picoeukaryotes				
Month	2	0.118	3.332	0.038
Shelf	2	0.094	2.663	0.073
Month*Shelf	4	0.124	3.502	0.009
Error	171	0.035		