Interactions between sponges and the water column: nutrient utilisation and feeding by New Zealand subtidal sponges

---

A thesis
submitted to the Victoria University of Wellington
in partial fulfilment of the requirements
for the Degree
of
Doctor of Philosophy in Marine Biology
by

Alejandra Perea-Blázquez

Victoria
UNIVERSITY OF WELLINGTON
Te Whare Wānanga
o te Ūpoko o te Ika a Māui

Victoria University of Wellington
2011

This thesis was conducted under the supervision of:

Dr. James J. Bell (Primary Supervisor)
Victoria University of Wellington,
Wellington, New Zealand

and

Dr. Simon K. Davy (Secondary Supervisor)
Victoria University of Wellington,
Wellington, New Zealand

This thesis is dedicated to my mother, my father and Benjamin who stood by me and believed that I could do it

*This photograph of a golf-ball sponge (*Tethya *sp.) was taken while diving at Breaker Bay, New Zealand on a good visibility day.*

Table of Contents

List of Figures

List of Tables

Abstract

Sponges are an important component of New Zealand subtidal communities and play many key functional roles in marine ecosystems, including competition for space, facilitating primary production, nutrient cycling, bioerosion, and interactions with the water column. Sponges are involved in the bidirectional movement of detritus, nutrients, micro-organisms and planktonic particles both to and from the benthos to the pelagic ecosystem, thereby affecting pelagic processes. As suspension-feeders, sponges are capable of filtering large volumes of water, and they depend on food that is suspended in the water column, meaning that their interaction with the water column is likely to be very important. The main goal of my research was to investigate the interactions between sponges and the water column and how this varies in relation to sponge characteristics, nutrient fluxes, seasonality and food supply. I studied the diet composition of 10 sponge species that are abundant and widely distributed along the south coast of Wellington, New Zealand. I found that the diet of the sponge species analysed comprised three types of picoplanktonic organisms: heterotrophic bacteria, Prochlorococcus, and Synechococcus. These micro-organisms (picoplankton) that sponges feed on are vital for benthic food webs because they are involved in the transformation and cycling process of dissolved inorganic nutrients before they become available to other marine organisms. The results from this thesis demonstrated that different sponge species have different retention efficiencies for different types of picoplankton and I propose that this suggests intra-phyletic food particle niche partitioning among sponges. While these findings support the partitioning of food resources between different co-existing sponge species, they also suggest that partitioning may not be essential for co-existence, as some species had similar retention efficiencies implying an overlap in resource use. By measuring rates of carbon assimilation in the form of planktonic food particles, combined with data on a number of characteristics of the sponge species analysed, I found that sponge assemblages play a key role in the transfer of energy from the water column to the benthos. The results from this thesis indicate that there is a wide range of food concentrations in the rocky reefs where the study species are living, over which

retention rate, nutrient utilisation and carbon consumption varied temporally. This emphasises the importance of understanding temporal variation in productivity, and suggests that such variations are likely to have important implications for suspension-feeders. By integrating the feeding results with estimations of oxygen consumption rates, and the amount of carbon obtained from the different micro-organisms found in the water column, preliminary carbon budgets were created. These budgets were used to quantify the capacity of carbon obtained via heterotrophic suspension-feeding to support sponge metabolism, as well as infer the potential for this carbon to support other processes such as sponge growth and reproduction. Overall, this project was the first to consider the functional roles of sponges in New Zealand marine ecosystems and provided useful information on their ecological and biological importance. The large amounts of carbon that sponges transfer from the water column to the benthos, in conjunction with the other findings of my thesis, increase our understanding of the ecology of temperate sponges.

Acknowledgments

I would like to express my most sincere gratitude to all those people who helped me throughout the duration of this journey. This thesis reflects in many ways, the help and support of many people who influenced my life in New Zealand and this work. The completion of this thesis would have never been possible without the support of my supervisors, friends, colleagues, family, and institutions. I am most grateful to my supervisors, James Bell and Simon Davy for sharing with me their knowledge and experience of different aspects of marine biology. I thank them for their positive feedback and continuous orientation. I am grateful for James's guidance throughout the development of my project, for his patience and for his constant motivation. His advice and suggestions aided the writing of this thesis in numerous ways. I was pleased to have Simon as my co-supervisor and I am grateful for his constant help and support with lab-related analyses and experiments, also for his help on the interpretation of my data, and for his willingness to help me out with any difficulties of my thesis. I also wish to thank Nicole Phillips and Jonathan Gardner, who are other members of my thesis committee, for providing guidance and support when I needed it. I also acknowledge Stephen Hartley who provided statistical advice, and I am very grateful to Benjamin Magaña, Shane Geange and Alejandro Pérez who introduced me to the wonders of R and for their help with statistical analyses. I wish to thank Dr. Michelle Kelly and Jade Berman for their taxonomic assistance and Dr. Adele Pile for her invaluable support with the underwater sampling technique and flow cytometry training. I am most grateful to Kylie Price for sharing

with me her remarkable knowledge and passion for flow cytometry, and also for working side-by-side with me until we developed an adequate analysis technique for my seawater samples. Those long hours in the flow cytometry suite were an unexpected place for finding a new friend. I must also express a very warm thank you to Bionda Morelissen for spending endless hours and many days mastering the art of using the nutrient analyser. She was a great lab partner, who became a good friend. I thank my friends and colleague divers for their assistance in the field: Jade Berman, Daniela Díaz, Alejandro Pérez, Pelayo Salinas, César Cárdenas, Danelle Lekan, and Tamsen Byfield. A special recognition goes to Benjamin Magaña for his underwater photographs and videos, a part from being an excellent dive buddy. My gratitude goes also towards my fellow postgraduate students, office-friends and colleagues who helped me throughout my PhD: Bionda, Jade, Danelle, Tim, Philip, Emily, Tyler (Tyla), Shane, Monica, Rachel, Ursula and Heather. Thank you all for your help and for being a constant reminder that life has more than stressing out for your PhD. A very special and warm thank you goes towards Lindsay, Marisol, Andrea Varela, Daniela, Alba, Florecita, Andrea Rojas, Nataliya, Katja, Mariana, Anita, Ale (tocayo), Pelayo (Pilajo), Sergio (keko), Erasmo, Israel, Cesar and Enrique. Thank you all for your friendship, support, many laughs, great meals and relaxing times. Special thanks go to Andrea and Keko for making possible the long-distance printing, binding and dealing with the paperwork of this thesis and for being good trustworthy friends. I am grateful to all the institutions that provided the necessary financial support for this research: Victoria University of Wellington for providing funding and a PhD Scholarship, as well as two Faculty Grants and further financial assistance through the Hardship Fund. I am also grateful to Victoria University for providing a wonderful space at Victoria University Coastal Ecology Lab (VUCEL); the seaview from my desk inspired me throughout my PhD. Education NZ provided a Postgraduate Study Abroad Award for flow cytometry training, and the NZ Marine Sciences Society provided a conference travel fund to attend my first overseas conference in Spain. I also acknowledge Helen Kettles from the Department of Conservation for permitting fieldwork within the Taputeranga Marine Reserve (permit docDM-336985). Special recognition goes to Sandra Taylor,

Patricia Stein and Mary Murray from SBS staff for their smiles, friendship and for their constant willingness to help me out in any aspect related to my PhD or in any personal matter. I also wish to express my gratitude to VUW technicians who helped me with lab or field related aspects of my project, making my life easier: Joanne Davy, John Van der Sman, Daniel McNaughtan (Snout) and Mairead Murphy. A warm thank you goes to Liz Medford and Maria Goncalves-Rorke who gave me their unconditional support and friendship during my time in Wellington. I also express my gratitude to Shona De Sain for her help and support. Most of all, I would like to thank my partner Benjamin for his patience and for his personal and professional support at all times. He encouraged me throughout the whole course of my PhD, and kept me going during rough times with his characteristic optimism and calm attitude. Loving thanks go to my mother Alejandra, my father Carlos, my sisters Ana Karla y Mariana, my grandparents, Soco and other members of my family for constantly bringing positivity and motivation into my life, and for their never-ending support.

Chapter 1

Introduction, overview and thesis structure

1.1 Introduction

The coupling of particles between pelagic and benthic habitats is dominated by the activities of suspension-feeding, in which the organisms actively use energy to pump water that is then filtered to remove suspended particles; these particles are then consumed while undigested remains are deposited on the bottom (Dame & Olenin 2005). Suspension-feeders are found in both pelagic and benthic systems, and they function as an important part of an ecosystem's biomachinery by removing suspended matter and dissolved inorganic materials, excreting faeces and producing pseudofaeces, that contribute to nutrient cycling between the water column and the benthic habitats (Ostroumov 2005). Benthic suspension-feeders in shallow water marine ecosystems are capable of transferring large quantities of particles such as nutrients, plankton, organic matter and detritus from the overlying water column to the benthos (Pile & Young 2006). This process is known as bentho-pelagic coupling; a two way inter-linked process where detritus, nutrients, microorganisms and planktonic particles are moved both to and from the benthos to the pelagic ecosystem controlling and affecting pelagic processes (Bell 2008). Sponges are involved in this bidirectional movement of nutrients, which may also influence their distribution (Bell 2008).

Sponges are one of the most abundant and widespread benthic groups in tropical, temperate, polar and abyssal marine ecosystems (Gili & Coma 1998; Bell & Barnes 2001, 2003; Jiménez & Ribes 2007). Sponges are sedentary, suspension-feeding metazoans in which their entire body is specialised to effectively and rapidly remove food particles from the water column (Riisgård et al. 1993). Water enters through tiny pores in the sponge body wall (ostia) and leaves through much larger openings (oscula) (Vogel 1974). Following capture, food particles are moved into the mesohyl interior and are phagocytosed by amoeboid sponge cells called archaeocytes (Hentschel et al. 2006).

Sponges play many important functional roles in marine ecosystems (Wulff 2006). In a recent review, Bell (2008) described the functional roles of sponges of temperate, tropical and polar regions. The roles identified for temperate regions include: facilitating primary production, provision of microhabitat and sponge associations, enhanced predation protection, enhancing survival success of other species, range expansions and camouflage through association with sponges, sponges as a settlement substrate, and sponges as agents of biological disturbance. Similarly, Díaz and Rüztler (2001) identified six major aspects of the biology and ecology of sponges that make them an important component of coral reef ecosystems including their diversity, abundance, symbiotic associations with micro-organisms, ability as spatial competitors, mineralisation, cementation, consolidation and regeneration, and exchanges with the water column. Sponges also have other functional roles (Bell 2002; Bell & Barnes 2003; Wulff 2006; Bell 2007) including habitat provision, predation, chemical defence, nutrient cycling, nitrification, bioerosion, and substrate stabilisation (Wulff 2001; Bell 2002; Bell & Barnes 2003; Wulff 2006; Bell 2007).

1.2 Interactions between sponges and the water column

The physical properties of seawater allow living creatures and particulate matter to remain in suspension, thereby creating a niche for suspension-feeding. Phytoplankton and micro-organisms predominate in such suspended (planktonic) communities (Gili & Coma 1998), and suspension-feeders

consume these suspended micro-organisms along with detritus (Riisgård & Larsen 2000). Sponges, as suspension-feeders, depend on food that is suspended in the water column (Orejas et al. 2000) and have evolved mechanisms for capturing food that is highly diluted within the water mass and too small to be detected and captured individually (Gili & Coma 1998). Here, it is important to clarify that in sponge literature, the term retention or filtration rate refers to the rate at which particles are retained from the water pumped through the sponges' aquiferous system (Turon et al. 1997); the volume of water that passes through the sponges' aquiferous system per unit sponge weight and unit time is called water transport rate, and the pumping rate is the speed at which the water exits a sponges' osculum for a given duration (Turon et al. 1997).

Sponges are often one of the most conspicuous components of freshwater and marine benthic communities and have been previously shown to feed on pico- and ultraplankton (organisms < 5 µm including non-photosynthetic bacteria and photosynthetic prokaryotes) (Reiswig 1971b; Pile 1997), and virus-like particles (Hadas et al. 2006). There is also evidence that sponges are able to capture larger cells (up to 70 µm) such as phytoplankton (i.e. diatoms, coccolithophores, dinoflagellates) (Reiswig 1971b; Frost 1981; Yahel et al. 1998; Ribes et al. 1999). A study of two freshwater sponges in Lake Baikal, Russia showed that the sponge assemblages living in lakes feed on picoplankton (plankton < 2 µm) (Pile et al. 1997). Similarly, Pile & Young (2006) found that dense beds of hexactinellid sponges are capable of moving large amounts of ultraplankton from the pelagic community to the benthos resulting in the transfer of up to 55 mg C m^2 d^{-1} and 7.3 mg N m^2 d^{-1} from the water column to the benthos. This means that these sponges are most likely nutritionally dependent on ultraplankton and that the fluxes of carbon and nitrogen provided from these micro-organisms place sponges within a functional group of organisms that link the pelagic microbial food web to the benthos.

We currently have a poor understanding of the relationship between sponges and the water column, although given the volume of water that sponges can filter ($2 - 12$ ml water/cm^3 sponge min^{-1}) (Reiswig 1974, 1981; Pile 1997), their role in bentho-pelagic processes is likely to be of great im-

portance (Díaz & Rutzler 2001). A number of experimental studies in tropical seas and in the Mediterranean have quantified the diet and metabolic contribution of all types of plankton to several taxa of benthic-suspension feeders such as sponges, bivalves, bryozoans, and ascidians (e.g. Orejas et al. 2000). Several studies have focused on the feeding ecology of sponges (e.g. Pile et al. 1996; Pile 1997; Yahel et al. 2005), with only a few studies looking at the natural diet of marine sponges in temperate regions (Reiswig 1975a; Ribes et al. 1999), and to date, there is only one published study looking at sponge feeding of a New Zealand sponge species (*Polymastia croceus*) (Bell et al. 1999).

In a different nutrient uptake pathway, Yahel et al. (2003) studied the direct uptake of bulk dissolved organic matter (DOM) by a common coral reef sponge that occurs throughout the Indo-West Pacific. The sponge removed up to 26% (2 11 µmol C L^{-1}) of the dissolved and particulate organic carbon although it is still uncertain if the uptake of DOM is by the sponge or by the symbionts it harbours. It has also been shown that cyanobacterial symbioses are crucial in the nutrition and ecology of coral reef sponges (Wilkinson 1983). Some authors have recently identified a very interesting potential nutrient pathway, suggesting that sponges have the ability to efficiently capture and ingest virus-like particles from the water column (Marie et al. 1999; Marie et al. 2001; Hadas et al. 2006; Patten et al. 2006). Hadas et al. (2006) calculated that viruses are likely to represent only a small fraction of the carbon required for sponge respiration (550 µg C day^{-1}g WW sponge) but due to the ubiquity of these organisms, a significant amount of nutrients may be transported from virus particles to higher trophic levels via sponges and other marine suspension-feeders; this was quantified using flow cytometry. In the present study viruses were not quantified. The DNA-binding dye used in this study for bacteria identification (Hoechst 33342) is not bright enough to give a clear signal of viral nucleic acid, compared to brighter nucleic acid dyes such as SYBR Green-I (Marie et al. 1999; Shapiro 2003) and SYBR Gold (Chen et al. 2001) that could not be used in this study due to pragmatic (e.g. cost, budget) reasons.

Sponges are important components of benthic ecosystems in New Zealand (Grange 1979; Ayling 1983; Duckworth & Battershill 2001) and as

suspension-feeders they can filter large volumes of water with feeding efficiencies in the range of 75–99% on plankton of 0.1–70 µm in size (Reiswig 1971b, 1974; Pile et al. 1996; Ribes et al. 1999); this means that their interaction with the water column is likely to be very important. Although there is a considerable amount of information available describing the functional roles of sponges on coral reefs (e.g. Díaz & Rutzler 2001; Wulff 2001), there are far fewer studies focusing on the functional roles that sponges play in temperate regions. Very little is known about the New Zealand sponge fauna and sponge ecology compared with other temperate regions. Although there have been several studies that have focused on sponge morphology and behaviour in New Zealand (Bergquist & Sinclair 1967), work on some specific attributes of sponge ecology such as grazing and predation as agents of biological disturbance (Ayling 1980; Ayling 1981), and aspects of population dynamics like growth and bioactivity of sponges (Duckworth & Battershill 2001), the functional roles of New Zealand sponges remain relatively unknown.

1.3 Fluxes of dissolved inorganic nutrients; their interaction with benthic organisms and the water column

A previous study in the Cook Strait determined the availability of dissolved inorganic nutrients in surface waters and their influence on phytoplankton production (Bradford et al. 1986). Results of this study provide a range for the maximum and minimum concentrations of nitrate, nitrite, phosphorus and silicate for this study region and for a range of phytoplankton biomasses. Menge et al. (1997) evaluated the variation in nutrient concentrations between two sites (one site where the abundance of filter-feeders was low and the other where it was high) at different coastal rocky intertidal communities on the Oregon coast. These authors concluded that differences in community structure and dynamics were not associated with differences in hydrodynamic conditions, but instead with bottom-up effects of phytoplankton. Specifically, they suggested that differences in filter-feeder growth rates and abundance, trophic interactions, and perhaps prey recruitment rates may depend on different phytoplankton concentrations. Hatcher (1994) calculated the relative rates of nitrogen and phosphorus retention and turnover for six

benthic invertebrates in Western Australia (including two sponge species) by measuring CO_2 production, O_2 consumption rates, ammonium, phosphate, dissolved organic nitrogen and dissolved organic phosphorus. The two species of sponges showed a net adjusted mean uptake of DON and high rates of ammonium excretion (0.87, 0.98 µmol g DW^{-1}, respectively). These studies are relevant to my thesis as they provide quantitative measurements of concentrations (µmol L^{-1}) of the nutrients that I am interested in analysing, which I can use as background information to see if the levels found are comparable or within the same ranges.

1.4 Ecology of sponges

Seasonality in the supply of potential food material may be an important factor that conditions the life strategies of benthic organisms, to the point of limiting their growth and reproductive output (Orejas et al. 2000). For many sessile suspension-feeders, like ascidians and sponges, ambient currents can increase the rate at which water passes through the organism's body under natural conditions (Vogel 1977). This may enhance flow and feeding, and reduce the energy required for metabolic processes, lowering the energy required for food capture and leaving more energy available for growth and reproduction (Orejas et al. 2000).

Sponges are organisms with high morphological plasticity that have the capacity to establish a multitude of associations with a large variety of organisms (Taylor et al. 2007; Bell 2008; Wulff 2008). Some of these associates include a range of photosynthetic organisms, such as cyanobacteria and to lesser extent, dinoflagellates and macroalgae, and ammonium-oxidizing bacteria (Wilkinson 1992; Sarà et al. 1998; Osinga et al. 2001; Usher 2008). These associations provide the potential for sponges to assimilate carbon from a range of associated micro-organisms, besides the assimilated carbon from feeding on ultraplankton, phytoplankton, viruses, and DOM (Díaz & Ward 1997; Ribes et al. 1999; Bell 2008). The benefits of photosynthetic symbionts to the sponge include a contribution to nutrition of the sponge (Reiswig 1981; Wilkinson 1983; Díaz & Ward 1997; Yahel et al. 2003), stabilisation of the sponge skeleton (Rüztler 1985) and processing of metabolic waste products

(Hoffmann et al. 2009). Much of the nitrogen within the organic matter and detritus that sponges remove from the water column would be metabolised by sponge cells and microbial symbionts to amino-nitrogen; it is then presumably oxidised to nitrate by symbiotic, nitrifying bacteria (Corredor et al. 1988). Thus, associated micro-organisms may play an important role in the nutrient assimilation/uptake by sponges.

Jiménez & Ribes (2007) reported excretion rates of $3 - 13$ µmol DIN g DW min^{-1} by sponges examined under laboratory conditions and suggested that this value is indicative of the high activity of micro-organisms inside the sponge. In a recent study, Weisz et al. (2008) measured mesohyl density for a variety of high microbial (HMA) and low microbial abundance (LMA) species, and pumping rates for a select group of these sponges. Their results showed that LMA sponges moved larger volumes of water through their porous tissues allowing them to rapidly obtain small particulate organic matter (POM) that supplied the majority of their nutritional needs. In contrast, HMA sponges hosted tightly packed communities of micro-organisms with an aquiferous system that increases contact time between seawater and the sponge/microbial consortium that feeds on particulate organic matter (POM), dissolved organic matter and raw inorganic materials (Weisz et al. 2008).

1.5 The use of flow cytometry to analyse the natural diet of sponges

The development of new flow cytometry techniques in the last decade has provided a reliable technique to examine natural microbial populations. The use of this technique has offered a substantial reduction in the volume of sample required and in the effort to examine the sample (e.g. Gasol & Del Giorgio 2000; Vives-Rego et al. 2000). Previous work has already discriminated different cell populations of micro-organisms found in seawater by flow cytometry: cyanobacteria (*Prochlorococcus*, *Synechococcus*), Prochlorophytes and picoeukaryotes (Campbell et al. 1994; Pile et al. 1996). This technique has also been successfully applied to examine the diet and feeding ecology of freshwater (Pile et al. 1997) and marine demosponges (Pile 1997; Ribes et

al. 1999; Yahel et al. 2003; Pile 2005), and their role in influencing water column communities. These authors found that the study species obtained the majority of their daily carbon from prokaryotic cell-types similar to Reiswig's (1971b) findings for other species. The uptake of particulate organic carbon (POC) is unselective, meaning that sponges obtained carbon from the various types of micro-plankton proportional to their abundance in the water column. This technical improvement has allowed the scientific community to realise the importance of pico- and nanoplankton in marine ecosystems in terms of biomass and production.

1.6 Thesis structure

My PhD focuses on the functional role of sponges as suspension-feeders in a temperate rocky subtidal ecosystem on the south coast of Wellington, New Zealand. The primary goal of my research was to investigate the interactions between sponges and the water column and to determine how this varied in relation to sponge characteristics, seasonality, food and nutrient supply for various common sponge species. In Chapter 2, I investigated the natural diet of two common species of calcareous sponges using the technique of flow cytometry as a proof of concept. This chapter provides a detailed description of the study sites and the sampling methodology. Also the methods for the flow cytometry analysis and the set of flow cytometric experiments performed are explained in detail. Subsequently in Chapter 3, I investigated the natural diet of seven sponge species that co-exist on a subtidal rocky reef. In this chapter my aim was to determine if there were differences in the picoplanktonic organisms being consumed by different sponge species living in the same habitat to explore the suggestion of intra-phyletic resource partitioning in these organisms.

In Chapter 4 I analysed a number of characteristics of the same sponge species studied in Chapter 3 to determine the ecological importance of a temperate sponge assemblage with respect to its contribution to energy flow from the water column to the benthos, by measuring rates of carbon assimilation in the form of particulate matter (food particles). In Chapter 5 I was interested in relating the importance of sponges to nutrient dynamics

and their effect on water column nutrient concentrations. In this chapter, I investigated the nutrient fluxes of several sponge species in order to estimate the amount of nutrients that sponges remove from (or release into) the water column at the assemblage level, as well as to increase our understanding of the availability and utilisation of nutrients over time. In Chapter 6, I investigated the temporal variation of food particles and consumption of picoplankton by three common sponge species over a 2-yr period. Finally, in Chapter 7 the temporal results from Chapter 6 detailing the consumption of carbon by the study species from the picoplanktonic particles removed over time, were integrated into a carbon budget. These budgets were used to deduce the capacity of carbon obtained via heterotrophic suspension-feeding to support sponge metabolism, as well as infer the potential for this carbon to support other processes such as sponge growth and reproduction.

1.7 References

- Ayling A.L. (1980). Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine demospongiae. Biological Bulletin, 158, 271-282.

- Ayling A.L. (1983). Factors affecting the spatial distributions of thinly encrusting sponges from temperate waters. Oecologia, 60, 412-418.

- Ayling A.M. (1981). The role of biological disturbance in temperate subtidal encrusting communities. Ecology, 62, 830-847.

- Bell A.H., Bergquist P.R. & Battershill C.N. (1999). Feeding biology of *Polymastia croceus*. Memoirs of the Queensland Museum, 44, 51-56.

- Bell J.J. (2002). The sponge community in a semi-submerged temperate sea cave: Density, diversity and richness. Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I, 23, 297-311.

- Bell J.J. (2007). Contrasting patterns of species and functional composition of coral reef sponge assemblages. Marine Ecology-Progress Series, 339, 73-81.

- Bell J.J. (2008). The functional roles of marine sponges. Estuarine, Coastal and Shelf Science, 79, 341-353.

- Bell J.J. & Barnes D.K.A. (2001). Sponge morphological diversity: a qualitative predictor of species diversity? Aquatic Conservation-Marine and Freshwater Ecosystems, 11, 109-121.

- Bell J.J. & Barnes D.K.A. (2003). The importance of competitor identify, morphology and ranking methodology to outcomes in interference competition between sponges. Marine Biology, 143, 415-426.

— Bergquist P.R. & Sinclair M.E. (1967). The morphology and behaviour of larvae of some intertidal sponges. New Zealand Journal of Marine and Freshwater Research, 2, 426-437.

— Bradford J.M., Lapennas P.P., Murtagh R.A., Chang F.H. & Wilkinson V. (1986). Factors controlling summer phytoplankton production in greater Cook Strait, New Zealand. New Zealand Journal of Marine and Freshwater Research, 20, 253-279.

— Campbell L., Nolla H.A. & Vaulot D. (1994). The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. Limnology and Oceanography, 39, 954-961.

— Chen F., Lu J.R., Binder B.J., Liu Y.C. & Hodson R.E. (2001). Application of digital image analysis and flow cytometry to enumerate marine viruses stained with SYBR gold. Applied and Environmental Microbiology, 67, 539-545.

— Corredor J.E., Wilkinson C.R., Vicente V.P., Morell J.M. & Otero E. (1988). Nitrate release by Caribbean reef sponges. Limnology and Oceanography, 33, 114-120.

— Dame R.F. & Olenin S. (2005). Comparative Roles of Suspension-Feeders in Ecosystems. Kluwer Academic Publishers, Dordrecht, The Netherlands.

— Díaz M.C. & Rutzler K. (2001). Sponges: An essential component of Caribbean coral reefs. Bulletin of Marine Science, 69, 535-546.

— Díaz M.C. & Ward B.B. (1997). Sponge-mediated nitrification in tropical benthic communities. Marine Ecology-Progress Series, 156, 97-107.

— Duckworth A.R. & Battershill C.N. (2001). Population dynamics and chemical ecology of New Zealand demospongiae *Latrunculia* sp nov and *Polymastia croceus* (Poecilosclerida : Latrunculiidae : Polymastiidae). New Zealand Journal of Marine and Freshwater Research, 35, 935-949.

— Frost T.M. (1981). Analysis of ingested particles within a fresh-water sponge. Transactions of the American Microscopical Society, 100, 271-277.

— Gasol J.M. & Del Giorgio P.A. (2000). Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina, 64, 197-224.

— Gili J.M. & Coma R. (1998). Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution, 13, 316-321.

— Grange K.R. (1979). Soft-bottom macrobenthic communities of Manukau Harbor, New Zealand. New Zealand Journal of Marine and Freshwater Research, 13, 315-329.

— Hadas E., Marie D., Shpigel M. & Ilan M. (2006). Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography, 51, 1548-1550.

— Hatcher A. (1994). Nitrogen and phosphorus turnover in some benthic marine invertebrates - implications for the use of C-N ratios to assess food quality. Marine Biology, 121, 161-166.

— Hentschel U., Usher K.M. & Taylor M.W. (2006). Marine sponges as microbial fermenters. FEMS Microbiology Ecology, 55, 167-177.

— Hoffmann F., Radax R., Woebken D., Holtappels M., Lavik G., Rapp H.T., Schlappy M.L., Schleper C. & Kuypers M.M.M. (2009). Complex nitrogen cycling in the sponge *Geodia barretti*. Environmental Microbiology, 11, 2228-2243.

— Jiménez E. & Ribes M. (2007). Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges. Limnology and Oceanography, 52, 948-958.

— Marie D., Brussaard C.P.D., Thyrhaug R., Bratbak G. & Vaulot D. (1999). Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied Environmental Microbiology, 65, 45-52.

— Marie D., Partensky F., Vaulot D. & Brussaard C. (2001). Enumeration of phytoplankton, bacteria, and viruses in marine samples. Current protocols in cytometry, Chapter 11, Unit 11.11.

— Menge B.A., Daley B.A., Wheeler P.A., Dahlhoff E., Sanford E. & Strub P.T. (1997). Benthic-pelagic links and rocky intertidal communities: Bottom-up effects on top-down control? Proceedings of the National Academy of Sciences, 94, 14530-14535.

— Orejas C., Gili J.M., Arntz W.E., Ros J.D., Lopez P.J., Teixido N. & Filipe P. (2000). Benthic suspension feeders, key players in Antarctic marine ecosystems? Contributions in Science, 1, 299-311.

— Osinga R., Armstrong E., Burgess J.G., Hoffmann F., Reitner J. & Schumann-Kindel G. (2001). Sponge-microbe associations and their importance for sponge bioprocess engineering. Hydrobiologia, 461, 55-62.

— Ostroumov S. (2005). Suspension-feeders as factors influencing water quality in aquatic ecosystems. Comparative Roles of Suspension-Feeders in Ecosystems, 47, 147-164.

— Patten N.L., Seymour J.R. & Mitchell A.G. (2006). Flow cytometric analysis of virus-like particles and heterotrophic bacteria within coral-associated reef water. Journal of the Marine Biological Association of the United Kingdom, 86, 563-566.

— Pile A.J. (1997). Finding Reiswig's missing carbon: quantification of sponge feeding using dual-beam flow cytometry. In: Proceedings of the 8th International Coral Reef Symposium, pp. 1403-1410.

— Pile A.J. (2005). Overlap in diet between co-occurring active suspension feeders on tropical and temperate reefs. Bulletin of Marine Science, 76, 743-749.

— Pile A.J., Patterson M.R., Savarese M., Chernykh V.I. & Fialkov V.A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone .2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnology and Oceanography, 42, 178-184.

— Pile A.J., Patterson M.R. & Witman J.D. (1996). *In situ* grazing on plankton <10 um by the boreal sponge *Mycale lingua*. Marine Ecology-Progress Series, 141, 95-102.

— Pile A.J. & Young C.M. (2006). The natural diet of a hexactinellid sponge: Benthic-pelagic coupling in a deep-sea microbial food web. Deep-Sea Research Part I-Oceanographic Research Papers, 53, 1148-1156.

— Reiswig H.M. (1971b). Particle feeding in natural population of 3 marine demosponges. Biological Bulletin, 141, 568-591.

— Reiswig H.M. (1974). Water transport respiration and energetics of 3 tropical marine sponges. Journal of Experimental Marine Biology and Ecology, 14, 231-249.

— Reiswig H.M. (1975a). Bacteria as food for temperate-water marine sponges. Canadian Journal of Zoology, 53, 582-589.

— Reiswig H.M. (1981). Partial carbon and energy budgets of the bacterio sponge *Verongia fistularis* Porifera Demospongiae in Barbados West-Indies. Marine Ecology, 2, 273-294.

— Ribes M., Coma R. & Gili J.M. (1999). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Riisgård H.U. & Larsen P.S. (2000). Comparative ecophysiology of active zoobenthic filter feeding, essence of current knowledge. Journal of Sea Research, 44, 169-193.

— Riisgård H.U., Thomassen S., Jakobsen H., Weeks J.M. & Larsen P.S. (1993). Suspension feeding in marine sponges *Halichondria panicea* and *Haliclona urceolus* - effects of temperature on filtration rate and energy cost of pumping. Marine Ecology-Progress Series, 96, 177-188.

— Rüztler K. (1985). Associations between Caribbean sponges and photosynthetic organisms. In: New Perspectives in Sponge Biology (ed. Rüztler K). Smithsonian Institution Press Washington, D.C., USA, pp. 455-466.

— Sarà M., Bavestrello G., Cattaneo-Vietti R. & Cerrano C. (1998). Endosymbiosis in sponges: Relevance for epigenesis and evolution. Symbiosis, 25, 57-70.

— Shapiro H.M. (2003). Practical flow cytometry. John Wiley & Sons, Inc., Hoboken, New Jersey.

— Taylor M.W., Radax R., Steger D. & Wagner M. (2007). Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiology and Molecular Biology Reviews, 71, 295-347.

— Turon X., Galera J. & Uriz M.J. (1997). Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. Journal of Experimental Zoology, 278, 22-36.

— Usher K.M. (2008). The ecology and phylogeny of cyanobacterial symbionts in sponges. Marine Ecology-an Evolutionary Perspective, 29, 178-192.

— Vives-Rego J., Lebaron P. & Caron G.N.-v. (2000). Current and future applications of flow cytometry in aquatic microbiology. FEMS Microbiology Reviews, 24, 429-448.

— Vogel S. (1974). Current-induced flow through sponge, *Halichondria*. Biological Bulletin, 147, 443-456.

— Vogel S. (1977). Current-induced flow through living sponges in nature. Proceedings of the National Academy of Sciences of the United States of America, 74, 2069-2071.

— Weisz J.B., Lindquist N. & Martens C.S. (2008). Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? Oecologia, 155, 367-376.

— Wilkinson C.R. (1983). Net primary productivity in coral reef sponges. Science, 219, 410-412.

— Wilkinson C.R. (1992). Symbiotic interactions between marine sponges and algae. In: Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored. (ed. Reisser W). Biopress Ltd. Bristol, England, pp. 113-151.

— Wulff J. (2001). Assessing and monitoring coral reef sponges: Why and how? Bulletin of Marine Science, 69, 831-846.

— Wulff J.L. (2006). Ecological interactions of marine sponges. Canadian Journal of Zoology, 84, 146-166.

— Wulff J.L. (2008). Collaboration among sponge species increases sponge diversity and abundance in a seagrass meadow. Marine Ecology-an Evolutionary Perspective, 29, 193-204.

— Yahel G., Marie D. & Genin A. (2005). InEx- a direct *in situ* method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnology and Oceanography, 3, 46-58.

— Yahel G., Post A.F., Fabricius K., Marie D., Vaulot D. & Genin A. (1998). Phytoplankton distribution and grazing near coral reefs. Limnology and Oceanography, 43, 551-563.

— Yahel G., Sharp J.H., Marie D., Hase C. & Genin A. (2003). *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography, 48, 141-149.

Chapter 2

Diet composition of two temperate calcareous sponges: *Leucosolenia echinata* and *Leucetta* sp. from the Wellington South Coast, New Zealand

Abstract

Sponges are an important component of benthic ecosystems in New Zealand, and as suspension-feeders they can process large volumes of water, meaning that their interaction with the water column is likely to be of great importance. The diet composition of two common species of calcareous sponges (*Leucosolenia echinata* and *Leucetta* sp.) was investigated by identifying and quantifying food particles from water samples using flow cytometry, and estimating the removal efficiency for these species. The natural diet of both species included heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*, similar to that previously reported for demosponges. Significant differences were found in the number of picoplanktonic organisms present in ambient and exhalant water from both study species. *Prochlorococcus* and *Synechococcus* were removed with the highest efficiency irrespective of sponge species ($52 - 57\%$). Both species had similar overall removal efficiencies, but significant differences were detected in the removal rates of the three types of bacteria in each species. This study is the first to investigate the natural diet of calcareous sponges in temperate rocky subtidal reefs using flow cytometry and it provides evidence for differences in the diets of calcareous sponges and demosponges. Furthermore, it provides proof of concept for the use of flow cytometry to quantify the picoplankton consumed by sponges.

2.1 Introduction

Sponges, as suspension-feeders, depend on food in the water column (Orejas et al. 2000), and have evolved mechanisms to capture food that is highly diluted within seawater and too small to be detected and captured individually (Gili & Coma 1998). Benthic-suspension feeders in shallow-water marine ecosystems are capable of transferring large quantities of material from the overlying water column to the sea floor (Pile & Young 2006). The high abundance of sponges in many benthic habitats is therefore likely to result in a considerable interaction between sponges and the water column, with sponges being an important link to higher trophic levels (Bell 2008).

Although the functional roles of sponges on coral reefs (e.g. bioeroders, reef consolidators/stabilisers) have been well described (e.g. Díaz & Rüztler 2001; Wulff 2006; Bell 2008), there are far fewer studies focusing on the functional roles that sponges play in temperate regions. Furthermore, very little is known about the New Zealand sponge fauna and its ecology compared with other temperate regions, such as southern Australia and northern Europe. Sponges are important components of benthic ecosystems in New Zealand and due to their high abundance, high filtration capacity, and a heterogeneous diet, sponges may be important in influencing nutrient dynamics in New Zealand coastal ecosystems (Jiménez & Ribes 2007).

While the feeding ecology of tropical demosponges has been well studied (Pile et al. 1996; Pile 1997; Hanson et al. 2009), there are few studies examining the natural diet of marine sponges in temperate regions (Reiswig 1975; Ribes et al. 1999a), and to my knowledge only one study has estimated the removal efficiency of a calcareous sponge (Wilkinson et al. 1984). These authors investigated the removal efficiencies of four tropical sponges from Australia including one calcareous species (*Pericharax heteroraphis*), which was measured by collecting *in situ* water samples and analysing them using the pour-plate counting technique. The authors found that all study species efficiently filtered heterotrophic bacteria from the ambient water with removal efficiencies of between 98% and 99% for the demosponges, and 95.5% for the calcareous sponge (Wilkinson et al. 1984).

In the last two decades, flow cytometry has become a valuable tool in

aquatic and environmental microbiology, and has largely been used to determine cell numbers, cell-size distribution, and additional biochemical and physiological characteristics of individual cells from different populations of picoplankton (plankton < 2 µm in size) (Vives-Rego et al. 2000; Marie et al. 2001b). The prokaryotic fraction of natural marine pelagic communities is composed of both heterotrophic and autotrophic organisms (Marie et al. 1997). These micro-organisms have been discriminated using flow cytometry as follows: *Prochlorococcus* ($0.6-0.8$ µm size), photosynthetic organisms that harvest light using divinyl-chlorophylls *a* and *b* (Chisholm et al. 1992) and only emit red fluorescence; *Synechococcus* (1 µm size), photosynthetic prokaryotes characterised by the dual fluorescence of their pigments. The other major prokaryotic group is the diverse non-photosynthetic bacteria (ranging between $0.2-2$ µm in size), characterised from flow cytometry by the absence of photosynthetic pigments and therefore autofluorescence.

The aim of this study was to provide proof of concept for the use of flow cytometry to quantify the picoplankton consumed by sponges. For this purpose, I identified and quantified food particles from water samples collected *in situ* from two species of calcareous sponges, *Leucosolenia echinata* and *Leucetta* sp. These species are particularly abundant and widely distributed along the south coast of the Wellington region and therefore potentially important in the cycling of food particles between the water column and the overlying rocky subtidal habitats.

2.2 Methods

2.2.1 Study sites

Sponge picoplankton removal was examined at two sites within the Taputeranga Marine Reserve on the south coast of Wellington, New Zealand (Fig. 2.1). The south coast of Wellington is a high energy environment, with its tidal and oceanic flows strongly influenced by Cook Strait weather (Carter 2008); water temperatures range between 11 ^{o}C in winter to 16 ^{o}C in summer (Berman & Bell 2010). This dynamic marine environment supports high sponge abundance and diversity, particularly on the sides of gulleys and crevices, and underneath rocks, boulders and overhangs in the rocky subtidal

ecosystems that characterise this coast (Berman et al. 2008). Two widespread calcareous sponges, *Leucosolenia echinata* (Kirk, 1893) and *Leucetta* sp.[1], were selected for this investigation because they are very common in the study area and their well defined exhalant oscula reduce the risk of sampling error and make sampling *in situ* easier.

2.2.2 Sampling

Seawater samples were collected *in situ* by SCUBA between 7 and 10 m depth. Samples for flow cytometry were collected in March 2009 in pairs from four specimens of each species ($n = 4$ exhalant and $n = 4$ inhalant, from each species), following a similar sampling method described by (Pile et al. 1996; 1997). Sample collection consisted of water being slowly drawn from the inhalant water at a distance of $\sim$ 3 cm from the sponge ostia, and then from the exhalant water inside the oscular aperture using 5-ml sterile plastic syringes with blunt-ended needles (Fig. 2.2); care was taken to avoid contact with sponge tissue. After collection, water samples were fixed with glutaraldehyde (0.1% final concentration), frozen in liquid nitrogen and then stored at -80 °C until the final flow cytometric analysis, following the protocol described by Marie et al. (2001a) for natural seawater samples. In preparation for flow cytometric analysis, samples were thawed to room temperature, then stained with the DNA-specific dye Hoechst 33342 (0.2 µg ml^{-1} final concentration) for bacterial identification; samples were kept in the dark at room temperature for 1 hr prior to analysis.

2.2.3 Flow cytometry analysis

To quantify the picoplankton populations, seawater samples were analysed using a BD LSR II SORP (Special Order Research Product) cytometer equipped with five lasers (20 mW 355 nm UV, 50 mW 405 nm Violet, 100 mW 488 nm Blue, 150 mW 532 nm Green, and 40 mW 633 nm Red lasers), at the Malaghan Institute of Medical Research in Wellington. Prior to the analysis, the LSRII SORP was calibrated using BD Cytometer Setup and Tracking Beads (Cat No. 641319). The size discrimination of the cytometer

[1] species photos in Appendix A.1b and A.1c

Figure 2.1: Map of the south coast of Wellington showing the two study sites, Mermaids Kitchen and The Sirens, within Taputeranga Marine Reserve, Wellington, New Zealand.This map was compiled by Benjamin Magaña-Rodríguez on June 2009. Data sources: New Zealand Territorial Authority data, Wellington Digital Elevation Model image. Projection and Datum: NZMG 1949.

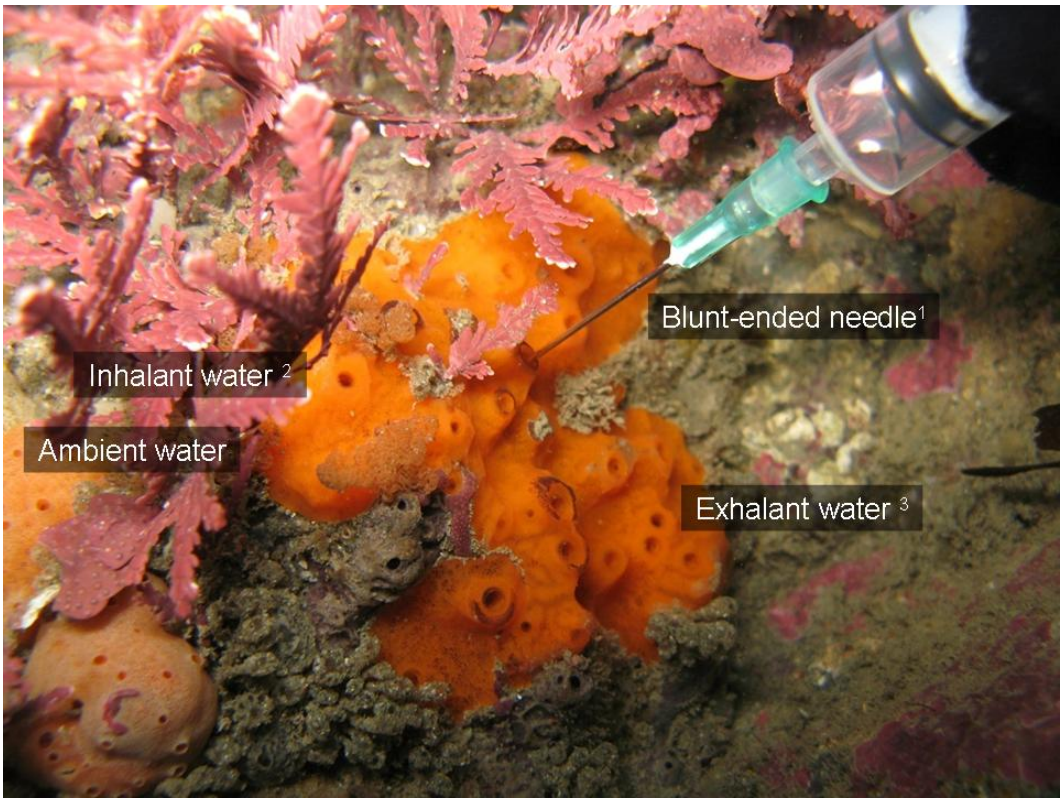


Figure 2.2: Schematic representation of *in situ* underwater sampling method. Water samples were collected using 5-ml syringes with blunt-ended needles[1]. Ambient water was collected from the inhalant water[2] at a distance of ∼3 cm from the sponge ostia, and the exhalant water[3] was collected from the oscular aperture.

was checked using yellow-green fluorescent microspheres (1 µm diameter). Forward Scattered Light (FSC) was collected using a photodiode and Side Scattered Light (SSC) was collected using a photomultiplier tube (PMT) with a 488 nm band-pass filter (488/10); due to the small size of the microorganisms the cytometer was set to trigger off SSC.

The DNA-specific dye Hoechst 33342 was excited using the 20 mW 355nm UV laser and the subsequent fluorescence was detected in the UV blue PMT through a 450/50 nm band-pass filter. The 100 mW 488 nm blue laser was used to excite chlorophyll and the emitted red fluorescence was passed through a 640 nm long-pass dichroic mirror and detected through a 685/35 nm band-pass filter. The 150 mW 532 nm green laser was used to better excite the phycobiliproteins and the emitted orange fluorescence was detected using a 575/26 nm band-pass filter. Two sets of flow cytometric experiments were carried out: The first set of experiments was performed in order to test the effectiveness of the sampling method. For this analysis, unstained (no Hoechst 33342 added) seawater samples from both study species were examined.

Cells of interest were identified based on being positive for Hoechst 33342 blue emission. DNA positive events were gated and viewed in a FSC vs SSC dot plot. A "cells of interest" gate was drawn, that included events falling above 10^2 on FSC and SSC to exclude noise. These events were viewed on a dot plot of orange fluorescence (y-axis) vs. red fluorescence (x-axis). Background noise was determined by running seawater through the cytometer to set an appropriate threshold and aid in gating.

Identification of all organisms of interest was initially based on the DNA gate. *Synechococcus* cells were identified based on both orange and red fluorescence emission; the phycobiliproteins contained in these organisms emit a strong orange fluorescence that can be detected separately from the red fluorescence emission of their chlorophyll (Campbell et al. 1994; Pile et al. 1996). *Prochlorococcus* cells were distinguished by the presence of red fluorescence and the lack of orange fluorescence. Non-photosynthetic bacteria were identified as being DNA positive events lacking both red and orange fluorescence. The non-photosynthetic microbes detected with the Hoechst staining as DNA containing particles were considered as bacterioplankton;

however, we do not know if these organisms are heterotrophic, chemosynthetic or chemoheterotrophic bacteria. These DNA containing particles are commonly referred as heterotrophic bacteria in the literature, therefore, and for simplicity's sake, these are hereafter referred to as heterotrophic bacteria. When using flow cytometry, heterotrophic bacteria can only be differentiated from *Prochlorococcus* by staining cells with specific nucleic acid fluorochromes e.g Hoechst 33342 (Marie et al. 1997). Finally, viruses ($0.002 - 0.2$ µm size fraction) are abundant, active components of aquatic ecosystems, but they are very difficult to quantify and classify (Vives-Rego et al. 2000). Despite these problems, some authors have been able to distinguish different populations of viruses in natural seawater samples (Marie et al. 1999; Marie et al. 2001a; Patten et al. 2006) using several fluorochromes (e.g. SYBR Green). In the present study viruses were not quantified.

Flow cytometry analysis was performed by `FlowJo` (version 8.8.6; Tree-Star, Ashland, OR), and data were presented using log-scale pseudo-colour dot plots for all parameters (SSC-A, FSC-A, orange fluorescence, red fluorescence). These plots showed three different populations of picoplanktonic organisms, *Synechococcus*, *Prochlorococcus* and heterotrophic bacteria that were mainly distinguished from the fluorescence emitted after excitation by the blue and green lasers.

2.2.4 Data analysis

A three-way ANOVA was used to determine if there were any significant differences between ambient and exhalant water cell concentrations for each type of picoplanktonic organism for each sponge species. In order to meet ANOVA assumptions of normality and homogeneity of variances, a normality test, and the Fligner-Killeen test (which is more robust than the Bartlett test and used when data seem not to be normally distributed) of homogeneity of variances were performed, respectively. When assumptions were not met, the data for cell counts were square-root transformed and arcsine transformed for the percentage data. An a posteriori Tukey test was carried out when significant results were obtained. The mean retention efficiency (the percentage of particles retained by the sponge filter) for the types of picoplankton

removed by each species was calculated using the following equation:

$$RE(\%) = \frac{Camb - Cexh}{Camb} * 100 \tag{2.1}$$

Where RE is the mean removal efficiency; $Camb$ is the mean cell count for ambient water; and $Cexh$ is the mean cell count for exhalant water (Pile 1997; Hanson et al. 2009); negative retention rates resulting from exhalant cell concentrations being higher than ambient ones, obtained from three specimens, were interpreted as no retention of picoplankton and consequently changed to zero. For comparisons between the removal efficiency of the two study species and the removal of the different picoplanktonic groups, a two-way ANOVA test was used. All statistical analyses were performed by `R` ver. 2.10 (R Development 6 Core Team 2011).

2.3 Results

The results from the first set of flow cytometric experiments (refer to the methods section) identified distinct populations of *Prochlorococcus* and *Synechococcus*. Once these populations could be clearly detected by the flow cytometer, the second set of experiments was performed using samples stained with Hoechst. The results from this analysis yielded a third population of picoplanktonic organisms, heterotrophic bacteria, and differences in the concentration of cells between ambient and exhalant water were observed in both sponge species (Fig. 2.3). As a result, the diet of *Leucosolenia echinata* and *Leucetta* sp. consisted of heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*. An extra population of picoplanktonic organisms appeared in the exhalant water samples from both species (Fig. 2.3). From the flow cytometric analysis, I suggest that in addition to background noise, there is an unidentified population of cells that may be different bacterial cells than the heterotrophic bacteria found in the ambient water, as they do not fit within the gating strategy used to analyse the populations of interest (*Prochlorococcus, Synechococcus* and heterotrophic bacteria).

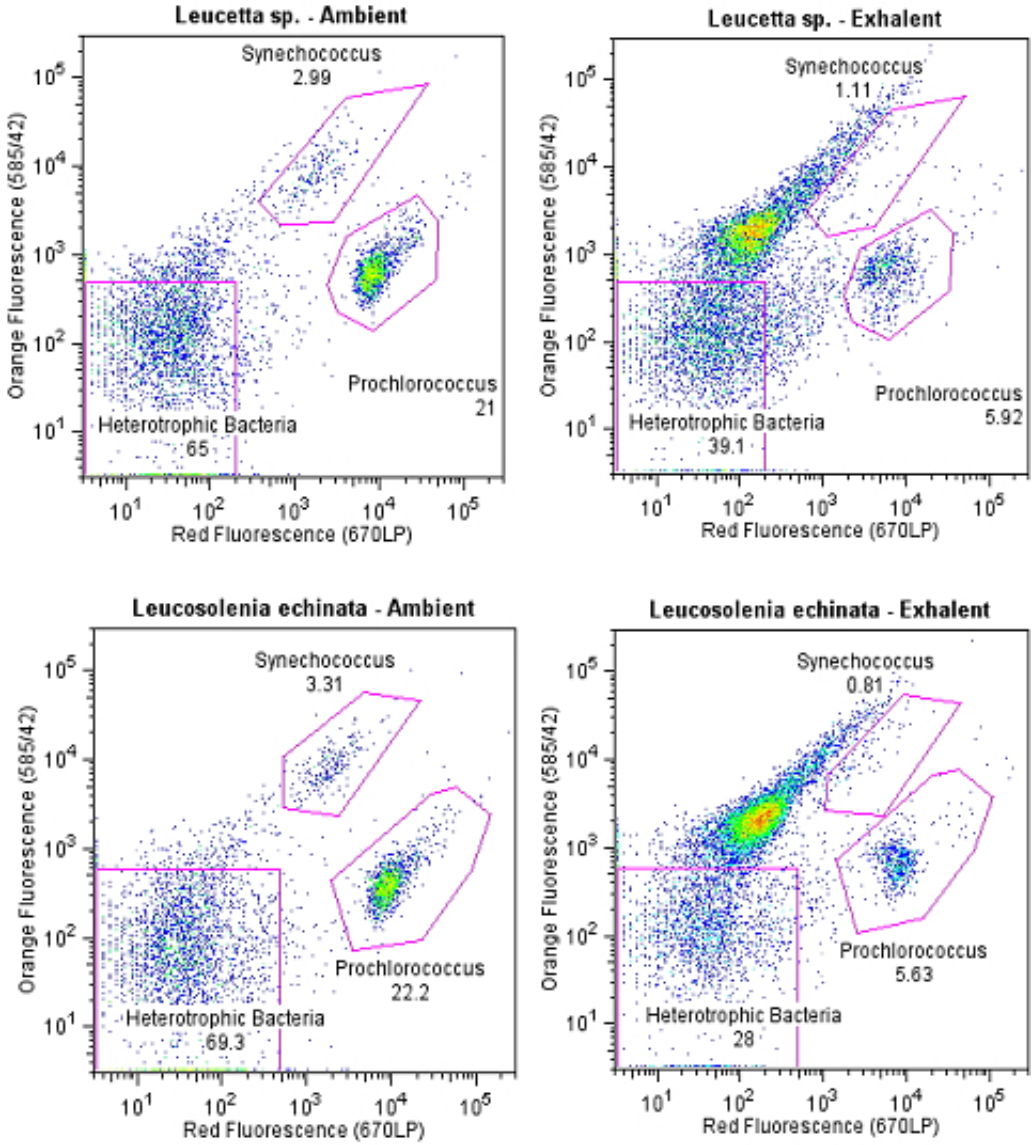


Figure 2.3: Flow cytometric analysis of seawater samples collected from two species of calcareous sponges on the south coast of Wellington, NZ. Pseudo-colour dot plots illustrating picoplanktonic populations of *Prochlorococcus*, *Synechococcus* and heterotrophic bacteria found in ambient and exhalant water samples that were identified based on the red and orange fluorescence emission profiles. DNA positive events were gated, based on a bright Hoechst 33342 blue emission, and then the populations of interest were analysed based on their orange and red emission as follows: *Synechococcus* exhibits bright orange and low red emission, *Prochlorococcus* displays bright red emission and no orange emission, and heterotrophic bacteria have no red or orange emission.

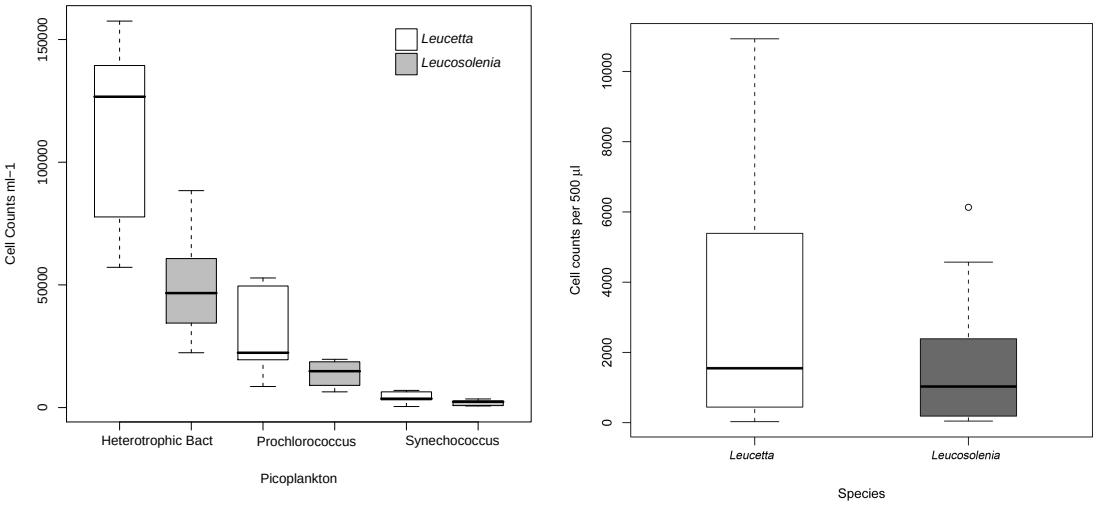
Table 2.1: Three-way analysis of variance showing differences in cell concentrations per 500 µl between *Leucosolenia echinata* and *Leucetta* sp., picoplanktonic organisms, and environment (ambient and exhalant water). Significant values in bold font.

Source of Variation	df	MS	*F value*
Species	1	3163.0	**32.43**
Picoplankton	2	13825.4	**141.74**
Environment	1	803.6	**8.23**
Species + Picoplankton	2	630.4	**6.46**
Species + Environment	1	35.1	0.36 NS
Picoplankton + Environment	2	1136.6	1.4 NS
Species + Picoplankton + Enviro	2	12.4	0.12 NS
Error	36	97.5	

2.3.1 Picoplanktonic concentrations in the water column

Significant differences were found in the number of picoplanktonic organisms between ambient and exhalant water from both *Leucosolenia echinata* and *Leucetta* sp. There were also significant differences in the cell counts between the different picoplanktonic organisms, and there was a significant difference between the sponge species and types of picoplankton (Table 2.1; Fig. 2.4a).

The overall concentration of heterotrophic bacteria ($8.1 \times 10^3 \pm 4.4 \times 10^3$ cells ml^{-1}) was significantly higher than *Prochlorococcus* ($2.2 \times 10^3 \pm 1.5 \times 10^3$ cells ml^{-1}) which was also significantly higher than *Synechococcus* ($3.1 \times 10^2 \pm 2.1 \times 10^2$ cells ml^{-1}) in both ambient and exhalant water from *Leucosolenia echinata* and *Leucetta* sp. (Fig. 2.4b). The a posteriori Tukey test showed that the cell counts of the picoplanktonic organisms were significantly higher in the ambient water surrounding both species (Tukey test $P < 0.001$). In addition, no significant differences were found in the concentrations of *Prochlorococcus* and *Synechococcus* in the ambient water surrounding both species (Tukey test $P > 0.05$), but the concentration of heterotrophic bacteria was significantly higher (Tukey test $P < 0.001$) in ambient water surrounding *Leucetta* sp. ($1.2 \times 10^5 \pm 4.2 \times 10^5$ cells ml^{-1}) than in the water surrounding *Leucosolenia echinata* ($5.2 \times 10^4 \pm 1.3 \times 10^4$ cells ml^{-1}) (Fig. 2.5).



(a) (b)

Figure 2.4: Box plots showing mean cell counts of (a) differences between each group of picoplanktonic organism (heterotrophic bacteria, *Synechococcus*, *Prochlorococcus*) measured for both sponge species, and (b) picoplanktonic organisms quantified from the ambient water surrounding both study species.

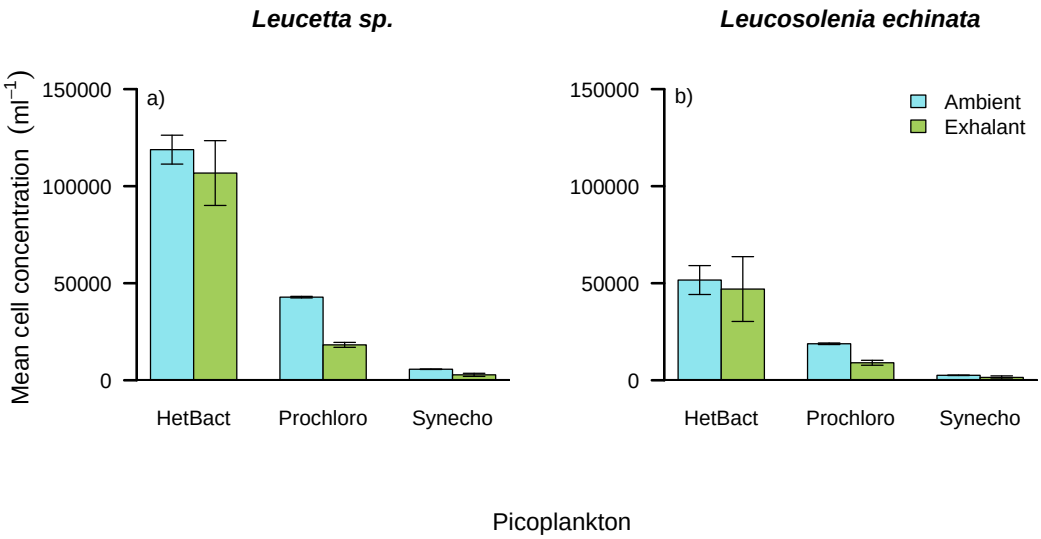


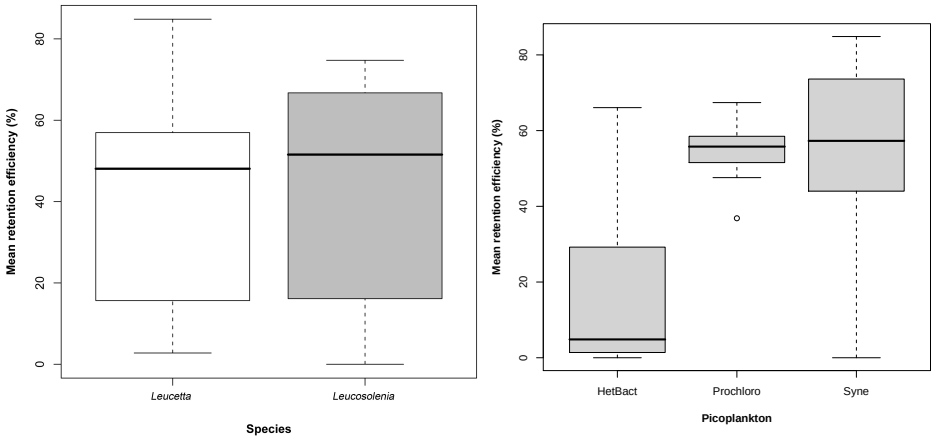
Figure 2.5: Mean cell counts (± StdDev) for each group of picoplanktonic organism found in ambient and exhalant water from *Leucosolenia echinata* and *Leucetta* sp.

Table 2.2: Two-way analysis of variance showing differences in the retention efficiency (percentage retained) of both sponge species and types of picoplankton. There was a significant difference in the removal efficiency of the three picoplanktonic organisms (heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*) by *Leucosolenia echinata* and *Leucetta* sp. Significant values in bold font.

Source of Variation	df	MS	*F value*
Species	1	5.1	0.89 NS
Picoplankton	2	2005.3	**6.46**
Species + Picoplankton	2	0.22	0.79 NS
Error	18	310.4	

2.3.2 Sponge prey removal

Both sponge species had similar overall removal efficiencies (two-way ANOVA, $F_{1,18} = 0.89$, $P > 0.05$), though significant differences in the removal rates of the three types of picoplankton by each species were detected (Table 2.2; Fig. 2.6). The a posteriori Tukey test revealed that the removal efficiency of heterotrophic bacteria ($10 - 25\%$) was significantly lower than that of *Prochlorococcus* ($52 - 57\%$) and *Synechococcus* ($53 - 55\%$) for both species (Fig. 2.7).



(a) (b)

Figure 2.6: Box plots showing mean retention efficiency expressed as (a) percentage of picoplankton retained by both sponge species and (b) percentage retained of each type of picoplanktonic organism per sponge species. HetBact-heterotrophic bacteria; Prochloro-*Prochlorococcus*; Syne-*Synechococcus*.

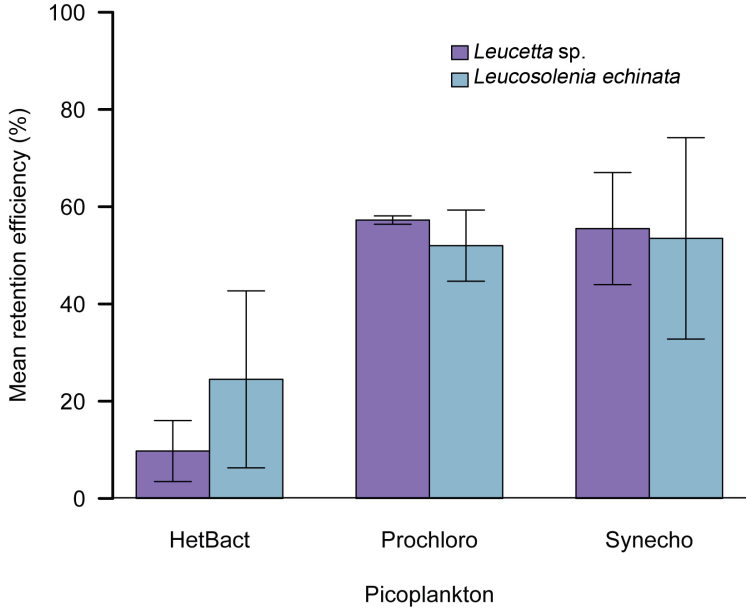


Figure 2.7: Retention efficiency (± StdDev) expressed as percentage removal of each group of picoplanktonic organism in *Leucosolenia echinata* and *Leucetta* sp.

2.4 Discussion

This study provides proof of concept for the use of flow cytometry to identify and quantify the picoplanktonic organisms consumed by sponges; as supported by the findings of the following chapters (Chapters 3 to 6). Furthermore, this is the first study to investigate the natural diet of calcareous sponges in a temperate rocky subtidal reef using flow cytometry. The results for the two calcareous species showed lower removal efficiencies, particularly of heterotrophic bacteria, and overall lower removal efficiencies of *Prochlorococcus* and *Synechococcus* compared to published data for other temperate demosponges (Pile 1997; Ribes et al. 1999a; Hanson et al. 2009). Also, a much higher concentration of heterotrophic bacterial cells was found in the ambient water around *Leucetta* sp. than in the water around *Leucosolenia echinata*, which was surprising as the water samples were collected on the same day from the same study site and type of habitat.

2.4.1 Removal efficiency of particles by calcareous sponges

Direct measurements have corroborated that both tropical and temperate sponges, as well as freshwater sponges, have high capture rates of picoplankton (Gili et al. 1984). The results from this study confirm the effectiveness of flow cytometry in analysing the dietary composition of sponges. The retention efficiencies of the two calcareous sponges studied ranged between 10 and 57%, for the same picoplanktonic organisms analysed in previous studies using flow cytometry. These removal efficiencies are lower than those obtained *in situ* from temperate demosponges in recent studies (Ribes et al. 1999a; Hanson et al. 2009), where removal efficiencies ranged between 60% and 90%; these are similar to the removal rates reported for tropical sponges (Pile 1997). The removal rates obtained may have been influenced, most importantly, by the fact that the study species are calcareous sponges and the majority of available data on removal rates are for demosponges. The possession of a skeleton made of calcium carbonate makes the Calcarea distinctive with respect to the other groups of sponges (Manuel et al. 2002) and in addition to their differences in spicule composition, water flow in calcareous sponges differs from demosponges due to their body construction

and aquiferous system (Manuel 2006). These characteristics might have an influence on the removal efficiencies and types of micro-organisms that these sponges filter from the water column, though specific mechanisms of how these characteristics translate to removal differences remain unknown (Leys & Eerkes-Medrano 2006; Yahel et al. 2006).

Francis and Poirrier (1986) studied the particle uptake of two freshwater sponges and suggested that particle selection is probably determined by the rate at which the sponge can degrade particles and derive nutrients from them, as well as by the general availability of picoplankton. Although the results presented here showed higher removal rates of *Prochlorococcus* and *Synechococcus* by both species, these percentages were lower than those obtained for other temperate demosponges. In their study of the metabolism of the calcareous sponge *Clathrina clathrus* from the Mediterranean, Burlando et al. (1992) suggested that biochemical variations (e.g. tissue sugar content, protein and lipid concentration) seemed to be related both to changes in seasonal feeding and to metabolic physiological responses caused by cyclic climatic events. These findings, in conjunction with those of Wilkinson et al. (1984), are relevant to this study as I propose that calcareous sponges may potentially degrade particles at lower rates than demosponges and that this difference may be related to physiological characteristics and complexity of the aquiferous system (Wehrl et al. 2007).

The retention efficiencies of heterotrophic bacteria measured in this study were between 10% and 25% in both study species. These values are markedly lower than those previously reported for temperate demosponges, where retention efficiencies of heterotrophic bacteria range from $43 - 90\%$ (Pile et al. 1996; Ribes et al. 1999b; Pile 2005; Yahel et al. 2005). In the work of Wilkinson et al. (1984), the percentage of heterotrophic bacteria removed by the calcareous sponge *Pericharax heteroraphis* was 95.5%. Although this species is a tropical sponge, it is the only removal efficiency value that has been measured for a calcareous sponge that can be compared with my results. It is possible that the low removal efficiencies obtained from the results presented here may have also been influenced by the time of year when samples were collected. The pattern of picoplankton retention by sponges may depend on the concentration of these particles present in the water column. A high con-

centration of picoplankton could imply that these sponges may only need to remove a small fraction of these particles in order to meet their nutritional requirements. On the other hand, total uptake may be influenced by the patchy distribution of the picoplankton in the water column; low nutrient concentrations in the water column; or short term physical events such as waves and storms that can affect food uptake and availability (Pile et al. 1996).

There is little information available on the ecology and physiology of calcareous sponges. Leys & Eerkes-Medrano (2006) investigated particle uptake in a calcareous sponge by feeding sponges with bacteria and latex microspheres, both *in situ* and *in vitro*. Although their study did not examine the retention rate of food particles, their results provide a detailed description of the physiological mechanisms of feeding in these sponges and the size of particles that they can effectively remove. These findings are relevant to this study as they observed uptake of 1-µm sized natural bacteria, similar to my findings where the removal of picoplanktonic organisms of 1 µm or less in size was measured from the ambient water as shown in the flow cytometric plots (see results section for more detail). In conclusion, I demonstrated that calcareous sponges consume on picoplankton (plankton < 2 µm), similar to data previously published for demosponges.

2.4.2 Additional population in the exhalant water

It is well known that sponges release bacterial cells different to the ones removed from the ambient water. The additional population that appeared in the exhalant water can be explained in light of the existing literature. There are some studies where authors have observed the production of particles in the exhalant water from different sponge species. These particles have been described as breakdown products of digestion called faecal-pellet-like aggregates (Stuart & Klum 1984; Wolfrath & Barthel 1989; Yahel et al. 2006), or as undigested particles released in membranous vacuoles (Willenz et al. 1986), or as intact cells (Hadas et al. 2009). Barthel & Wolfrath (1989) also found that other particles observed in the exhalant water from sponges were the result of the sloughing off of surface tissue in the form of flakes that consist

of sponge tissue, spicules, fragments of copepod carapaces, diatom frustules and sand grains. It is known that the internal tissue of marine sponges is the habitat of symbiotic bacteria (Vacelet 1975; Wehrl et al. 2007). Due to the high filtration rates of bacteria in sponges, it is likely that the sponge host exerts some control over its symbionts (Taylor et al. 2007). Sponges may expel symbionts when stressed or to regulate the number of bacteria within the sponge (Huysecom et al. 1988; Rüztler 1988). Wilkinson et al. (1984) in their study of four species of marine sponges, reported evidence that sponges are able to discriminate between bacteria normally found in the ambient water (regarded as food), and symbiotic bacteria isolated from the sponges themselves. They showed that symbiotic bacteria passed readily through the sponge, whereas seawater bacteria were retained. Similarly, Gili et al. (1984) reported that bacterial colonies growing in plates with exhaled water samples, presented different forms and colours than the bacterial colonies growing in plates with ambient water samples suggesting that the nature of the exhaled bacteria was different from the inhaled bacteria. In the case of the study species examined here, future work is needed to investigate whether or not the released cells are live micro-organisms (symbiotic bacteria or other types of bacteria), breakdown products or digestion or undigested particles.

To conclude, both sponge species studied are efficient feeders on *Synechococcus* and *Prochlorococcus* with removal efficiencies ranging between 55% and 57%, respectively. *Prochlorococcus* and *Synechococcus* cell-removal rates were higher than those for heterotrophic bacteria in both study species, suggesting a higher grazing efficiency upon these prey types and a markedly lower removal efficiency of heterotrophic bacteria. Comparison of removal efficiencies between species and classes is difficult due to the variability within each species and group (Ribes et al. 1999a). Future studies are needed to evaluate if the ambient heterotrophic bacterial concentrations change through time and if there is a correlation between the concentration of dissolved nutrients in the water and the concentration of these picoplanktonic organisms. These topics are studied and discussed in Chapters 5 and 6. Also, aspects of the nutritional ecology of calcareous and demosponges related to seasonality and availability of picoplanktonic populations in temperate ecosystems, are

yet to be described. These issues are examined in Chapters 4 and 6.

2.5 References

— Barthel D. & Wolfrath B. (1989). Tissue sloughing in the sponge *Halichondria panicea* - a fouling organism prevents being fouled. Oecologia, 78, 357-360.

— Bell J.J. (2008). The functional roles of marine sponges. Estuarine, Coastal and Shelf Science, 79, 341-353.

— Berman J. & Bell J.J. (2010). Spatial variability of sponge assemblages on the Wellington South Coast, New Zealand. Open Marine Biology Journal, 4, 00-00.

— Berman J., Perea-Blázquez A., Kelly M. & Bell J. (2008). Sponges of the Wellington south coast. In: The Taputeranga Marine Reserve (eds. Gardner J & Bell J). First Edition Ltd Wellington, pp. 225-236.

— Burlando B., Bavestrello G. & Arillo A. (1992). Seasonal changes in the metabolism of the calcareous sponge *Clathrina clatrhus* (Schmidt). Comparative Biochemistry and Physiology a-Physiology, 101, 341-344.

— Campbell L., Nolla H.A. & Vaulot D. (1994). The importance of *Prochlorococcu*s to community structure in Central North Pacific Ocean. Limnology and Oceanography, 39, 954-961.

— Carter L. (2008). Below low tide - a seabed in motion. In: The Taputeranga marine reserve (ed. Gardner J). First Edition Ltd Wellington, p. 532.

— Chisholm S.W., Frankel S.L., Goericke R., Olson R.J., Palenik B., Waterbury J.B., Westjohnsrud L. & Zettler E.R. (1992). *Prochlorococcus marinus* nov gen nov sp. - an oxyphototrophic marine prokaryote containing divinyl Chlorophyll a and chlorophyll b. Archives of Microbiology, 157, 297-300.

— Díaz M.C. & Rutzler K. (2001). Sponges: An essential component of Caribbean coral reefs. Bulletin of Marine Science, 69, 535-546.

— Francis J.C. & Poirrier M.A. (1986). Particle uptake in two fresh-water sponge species, *Ephydatia fluviatilis* and *Spongilla alba* (Porifera: Spongillidae). Transactions of the American Microscopical Society, 105, 11-20.

— Gili J.-M., Bibiloni M.A. & Montserrat A. (1984). Tasas de filtración y retención de bacterias "*in situ*" de tres especies de esponjas litorales. Estudio preliminar. Miscelánea Zoológica, 8, 13-21 (in Spanish).

— Gili J.M. & Coma R. (1998). Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution, 13, 316-321.

— Hadas E., Shpigel M. & Ilan M. (2009). Particulate organic matter as a food source for a coral reef sponge. Journal of Experimental Biology, 212, 3643-3650.

— Hanson C.E., McLaughlin M.J., Hyndes G.A. & Strzelecki J. (2009). Selective uptake of prokaryotic picoplankton by a marine sponge (*Callyspongia* sp.) within an oligotrophic coastal system. Estuarine Coastal and Shelf Science, 84, 289-297.

— Huysecom J., Richellemaurer E., Vandevyver G. & Vray B. (1988). Effect of bacterial concentration on retention and growth rate of the fresh water sponge *Ephydatia fluviatilis*. Physiological Zoology, 61, 535-542.

— Jiménez E. & Ribes M. (2007). Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges. Limnology and Oceanography, 52, 948-958.

— Leys S.P. & Eerkes-Medrano D.I. (2006). Feeding in a calcareous sponge: Particle uptake by pseudopodia. Biology Bulletin, 211, 157-171.

— Manuel M. (2006). Phylogeny and evolution of calcareous sponges. Canadian Journal of Zoology-Revue Canadienne De Zoologie, 84, 225-241.

— Manuel M., Borojevic R., Boury-Esnault N. & Vacelet J. (2002). Class Calcarea Bowerbank, 1864. In: Systema Porifera: A Guide to the Classification of Sponges (ed. Hooper JNA). Kluwer Academic/Plenum Publishers New York, p. 1708.

— Marie D., Brussaard C.P.D., Thyrhaug R., Bratbak G. & Vaulot D. (1999). Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied Environmental Microbiology, 65, 45-52.

— Marie D., Partensky F., Jacquet S. & Vaulot D. (1997). Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology, 63, 186-193.

— Marie D., Partensky F., Vaulot D. & Brussaard C. (2001). Enumeration of phytoplankton, bacteria, and viruses in marine samples. Current protocols in cytometry, Chapter 11, Unit 11.11.

— Marie D., Simon N., Guillou L. & Partensky F. (2000). DNA/RNA analysis of phytoplankton by flow cytometry. Curr Protoc Cytom, Chapter 11, Unit 11.12.

— Orejas C., Gili J.M., Arntz W.E., Ros J.D., Lopez P.J., Teixido N. & Filipe P. (2000). Benthic suspension feeders, key players in Antarctic marine ecosystems? Contributions in Science, 1, 299-311.

— Patten N.L., Seymour J.R. & Mitchell A.G. (2006). Flow cytometric analysis of virus-like particles and heterotrophic bacteria within coral-associated reef water. Journal of the Marine Biological Association of the United Kingdom, 86, 563-566.

— Pile A.J. (1997). Finding Reiswig's missing carbon: quantification of sponge feeding using dual-beam flow cytometry. In: Proceedings of the 8th International Coral Reef Symposium, pp. 1403-1410.

— Pile A.J. (2005). Overlap in diet between co-occurring active suspension feeders on tropical and temperate reefs. Bulletin of Marine Science, 76, 743-749.

— Pile A.J., Patterson M.R. & Witman J.D. (1996). *In situ* grazing on plankton <10 um by the boreal sponge *Mycale lingua*. Marine Ecology Progress Series, 141, 95-102.

— Pile A.J. & Young C.M. (2006). The natural diet of a hexactinellid sponge: Benthic-pelagic coupling in a deep-sea microbial food web. Deep-Sea Research Part I-Oceanographic Research Papers, 53, 1148-1156.

— R Development Core Team, 2011. R: A Language and Environment for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

— Reiswig H.M. (1975). Bacteria as food for temperate-water marine sponges. Canadian Journal of Zoology-Revue Canadienne De Zoologie, 53, 582-589.

— Ribes M., Coma R. & Gili J.M. (1999a). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Ribes M., Coma R. & Gill J.M. (1999b). Seasonal variation of particulate organic carbon, dissolved organic carbon and the contribution of microbial communities to the live particulate organic carbon in a shallow near-bottom ecosystem at the Northwestern Mediterranean Sea. Journal of Plankton Research, 21, 1077-1100.

— Rüztler K. (1988). Mangrove sponge disease induced by cyanobacterial symbionts - failure of a primitive immune system. Diseases of Aquatic Organisms, 5, 143-149.

— Stuart V. & Klumpp D.W. (1984). Evidence for food-resource partitioning by kelp-bed filter feeders. Marine Ecology Progress Series, 16, 27-37.

— Taylor M.W., Radax R., Steger D. & Wagner M. (2007). Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiology and Molecular Biology Reviews, 71, 295-347.

— Vacelet J. (1975). Electron-microscope study of association between bacteria and sponges of genus *Verongia* (Dictyoceratida). Journal De Microscopie Et De Biologie Cellulaire, 23, 271-&.

— Vives-Rego J., Lebaron P. & Caron G.N.-v. (2000). Current and future applications of flow cytometry in aquatic microbiology. FEMS Microbiology Reviews, 24, 429-448.

— Wehrl M., Steinert M. & Hentschel U. (2007). Bacterial uptake by the marine sponge *Aplysina aerophoba*. Microbial Ecology, 53, 355-365.

— Wilkinson C.R., Garrone R. & Vacelet J. (1984). Marine sponges discriminate between food bacteria and bacterial symbionts - electron-microscope autoradiography and *in situ* evidence. Proceedings of the Royal Society of London Series B-Biological Sciences, 220, 519-528.

— Willenz P., Vray B., Maillard M.P. & Vandevyver G. (1986). A quantitative study of the retention of radioactively labeled *Escherichia coli* by the fresh water sponge *Ephydatia fluviatilis*. Physiological Zoology, 59, 495-504.

— Wolfrath B. & Barthel D. (1989). Production of fecal pellets by the marine sponge *Halichondria panicea* Pallas (1766). Journal of Experimental Marine Biology and Ecology, 129, 81-94.

— Wulff J.L. (2006). Ecological interactions of marine sponges. Canadian Journal of Zoology, 84, 146-166.

— Yahel G., Eerkes-Medrano D.I. & Leys S.P. (2006). Size independent selective filtration of ultraplankton by hexactinellid glass sponges. Aquatic Microbial Ecology, 45, 181-194.

— Yahel G., Marie D. & Genin A. (2005). InEx - a direct *in situ* method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnology and Oceanography, 3, 46-58.

Chapter 3

Differential food resource utilisation enables co-existence of sponges in a temperate rocky reef system: a potential mechanism for niche partitioning

Abstract

Understanding the way that co-existing species utilise resources is important since such relationships are generally the result of long-term evolutionary processes. Key to understanding co-existence is the concept of resource partitioning among the constituent species of a community. In marine ecosystems, the utilisation or segregation of food resources based on food type has been investigated in order to determine if niche partitioning can explain the co-existence of ecologically similar species. In this chapter, I used seven marine sponges (*Dysidea* sp., *Haliclona* sp., *Plakina* sp., *Polymastia* sp., *Tethya bergquistae*, *Leucetta* sp. and *Leucosolenia echinata*) to study niche partitioning among members of a sessile-benthic assemblage. Under the premise that sponges can remove different sized food particles from the water they filter, I aimed to determine if differences exist in the picoplanktonic organisms being consumed by different sponge species living in the same habitat, and therefore suggest intra-phyletic niche partitioning as a possibility to explain species co-existence. The results from this chapter demonstrate that different sponge species have different retention efficiencies for different types of picoplankton and I propose that this could be indicative of intra-phyletic food particle niche partitioning among

sponges. The variability in retention efficiency among sponge species seems to be influenced by a combination of different factors including particle size and food concentration; however, there was no consistent pattern for all species. While these findings support the partitioning of food resources among different co-existing sponge species, they also suggest that partitioning may not be essential for co-existence, as some species had similar retention efficiencies implying an overlap in resource use. This intra-phyletic difference in trophic ecology may be one of the main factors contributing to the worldwide success of sponges despite the large spatial and temporal variation in sponge food availability.

3.1 Introduction

Darwin (1859) was among the first to recognise the importance of niche partitioning when stating that natural selection can only operate when there are 'places which can be better occupied by the modification of existing inhabitants, and as old inhabitants become modified, the mutual relations of others will often be disturbed; this will create new places ready to be filled up by better adapted forms...'(see Patten & Auble 1981). Since this time, the term 'niche' has been used in a variety of contexts by ecologists intrigued with habitat and life history differences of closely-related species (Flint & Kalke 1986). In 'conventional' niche theory resources become available and are consumed by organisms in proportions that depend on the occurrence and density of other species (Leibold 1995). Because species partition the limiting resources (e.g. nutrients, water, and light) in such a way that each is limited by a different amount of the available resources (Shmida & Ellner 1984), the co-existence of similar species within the same ecosystem may occur as a result of different resource use (Sala & Ballesteros 1997). Once differences in resource use between species are established, it has been suggested that this will lead to resource partitioning, mediating species co-existence and eventually promoting biodiversity; however, the evolutionary and ecological factors that lead to differences in resource use remain controversial (Tilman 2004; Bastolla et al. 2005).

Species do not exist in isolation and in most environments many species co-exist, and it is highly likely that closely related species may depend on the same or similar resources (e.g. food and space). Understanding the way that co-existing species utilise resources is important, since such relationships are

generally the result of long-term evolutionary processes. Furthermore, understanding how organisms share resources may provide an increased ability to determine how communities will respond to future changes in these resources. Key to understanding co-existence is the concept of resource partitioning among constituent species, where species may use different subsets of the total resource pool (Northfield et al. 2010). It is also possible for species to co-occur without any evidence of niche differentiation, with alternatives to niche theory being able to explain this co-existence (see Genner et al. 1999).

Benthic-suspension feeders are recognised as being important components of shallow marine ecosystems, where they are capable of moving large quantities of particles from the water column to the benthos (Ribes et al. 2005; Pile & Young 2006). These organisms, through their suspension-feeding activities, affect water clarity, productivity, and bentho–pelagic coupling, a two way inter-linked process where detritus, nutrients, micro-organisms and planktonic particles are moved between the benthos and the pelagic ecosystem, thereby controlling and affecting pelagic processes and vice versa (Menge et al. 1997; Gili & Coma 1998). In most rocky benthic environments a large number of different suspension-feeders occur in the same habitat. The co-existence of closely related members (e.g. same phylum) of a community suggests some kind of resource partitioning to help reduce potential intra-phyletic competition (Stuart & Klumpp 1984). In benthic communities, previous analyses have provided evidence for competition, which results in niche separation and the co-existence of ecologically similar species (Hines 1982). For example, sponges, ascidians, and bivalves reduce inter-phyletic competition by partitioning their food resources based on different sized particles or by filtering water from different heights above the substratum (Stuart & Klumpp 1984).

A number of experimental studies in tropical and temperate environments have quantified the particle uptake and metabolic contribution of all types of plankton to the diets of different benthic suspension-feeding taxa, including sponges, bivalves, bryozoans, tunicates, corals and ascidians (Gili & Coma 1998; Orejas et al. 2000; Pile 2005; Ribes et al. 2005). The main dietary components of these different groups consist of dissolved organic matter (DOM), bacteria and phytoplankton, although the uptake efficiency and

capture rates of the different types of food differ within the specific groups (Gili & Coma 1998). For example, tropical, temperate and deep-sea sponge species show considerable variation in retention efficiencies for different prey sizes (Reiswig 1971b; Pile et al. 1996; Turon et al. 1997; Witte et al. 1997; Ribes et al. 1999a).

In this study, seven sponge species (five demosponges and two calcareous) were used as a model to study niche partitioning in a sessile-benthic assemblage given that, among the benthic-suspension feeders, sponges are one of the most abundant and widespread groups and they perform many important functional roles within the marine environment (Wulff 2006; Bell 2008). In spite of the ecological importance of sponges, there have been few studies looking at resource partitioning in this group. Early work by Hartman (1957) introduced the concept of ecological niche differentiation for marine sponges by studying the distribution and habitat occupancy of nine species of boring (Clionidae) sponges. He concluded that competition for substrata was very important in determining the species' distribution patterns and suggested that differences in food preferences may exist between the species that might be significant enough to allow species to co-exist. In support of this concept, when studying the population dynamics, rates of water transport, oxygen consumption and energy cycling of three tropical sponge species, Reiswig (1973, 1974) suggested that niche partitioning within the Porifera occurred through the interaction of many factors including the life cycle of the species, energy channelling, and reproduction. He concluded that unlike most other animal groups, niche partitioning in sponges appears not to be accomplished by direct food specialisation since he only detected a significant dietary difference for one species, compared to the other two, which he attributed to the activity of bacterial symbionts. In a recent study of two co-existing species of glass sponges (Hexactinellidae) from a temperate fjord, Yahel et al. (2007) found a clear difference in food flux and pumping rates between the two sponge species suggesting that niche partitioning was occurring between the two species. Finally, in a study of four species of Antarctic sponges Thurber (2007), using stable isotopes and fatty acid analyses, demonstrated for the first time that co-occurring sponges exhibit niche separation in their diet.

There is a long-standing debate concerning the ability of species to parti-

tion resources at fine scales and the potential for species that are 'ecologically indistinguishable' to co-exist (Genner et al. 1999). Despite this debate, previous research investigating differences in diet and food resource utilisation in co-existing aquatic species has highlighted the importance of food partitioning as an important co-existence mechanism (e.g. Ross 1986; Genner et al. 1999; Martin & Genner 2009). In marine ecosystems, the utilisation or segregation of food resources based on food type has also been investigated in order to determine if niche partitioning alone can explain the co-existence of ecologically similar species, or if there are other niche dimensions to consider (Hines 1982; Flint & Kalke 1986; Sala & Ballesteros 1997). Under the premise that sponges can remove different sized food particles from the water that they filter, I re-examined Reiswig's (1973, 1974) suggestion that niche partitioning in sponges is not accomplished by feeding specialisations or selective food fractionation. For this purpose, I investigated the natural diet of seven sponge species (five demosponges and two calcareous sponges) that co-exist on a subtidal rocky reef. I aimed to determine if differences exist in the picoplanktonic organisms being consumed by different sponge species living in the same habitat, and therefore provide evidence for intra-phyletic niche partitioning in these organisms.

3.2 Methods

The study sites are located within the Taputeranga Marine Reserve on the south coast of Wellington in New Zealand (see Chapter 2 for more details). Sampling was conducted from November 2008 to March 2009. This time period for sampling reflects the difficulty in sampling along this coast as it is a very dynamic environment. Samples from each species were haphazardly collected across this time period in case of any seasonal bias. The subtidal rocky reefs along this coast support a high diversity and abundance of sponge species that are particularly found on the sides of channels, crevices, rock walls, boulders and overhangs that are common in the area (Berman & Bell 2010).

3.2.1 Sampling and flow cytometry

All samples were collected *in situ* by diving at a maximum depth of 10 m, and analysed using flow cytometry. Previous surveys have described the sponge biodiversity and abundance in the study area (Berman et al. 2008; Berman & Bell 2010) and from these data seven of the most common and widespread sponge species were selected including: *Dysidea* sp., *Haliclona* sp., *Plakina* sp., *Polymastia* sp., *Tethya bergquistae* (Hooper, 1994), *Leucetta* sp., and *Leucosolenia echinata* (Kirk, 1893). The species were chosen because they are all common encrusting species in the study area and because of their well-defined exhalant oscula that facilitate *in situ* sampling. *Dysidea* sp. is often found on rock walls or on the sides of channels; it can be lilac or brown-beige coloured and has a conulose surface with several cone-shaped projections raised up by underlying skeleton (Boury-Esnault & Rützler 1997). *Haliclona* sp. is commonly found on rock walls and overhangs; it has a digitate surface and is a white-cream colour, sometimes with finger-like outgrowths arising from the basal mass (Boury-Esnault & Rützler 1997). *Plakina* sp. is a very conspicuous encrusting species found on boulders, rock walls, crevices and overhangs; it has a bright violet-blue colour and a lobed smooth surface. *Polymastia* sp. is commonly found on rock walls and channels and has a very distinctive papillate surface with an orange-yellow colour. *Tethya bergquistae* is a pink ball sponge with 3−4 oscula; the surface can be rounded and smooth-to-warty in appearance. *Leucosolenia echinata* is a very common calcareous sponge mostly found on rock walls and crevices in the subtidal zone. It is easily recognisable as a mass of short white tubes, often with a brown rim at the top of the tubes. *Leucetta* sp. is another calcareous sponge that is widely distributed and mainly found on rock walls; it is cream coloured but can look pink-orange because of the red algae living on the sponges' surface. This calcareous sponge grows about five centimetres high and has well defined oval oscula (see species photos in Appendix A).

The selected species represented $20-25\%$ of the total sponge abundance in the rocky habitats of the Wellington south coast. Three specimens of each species were haphazardly selected and water was sampled from each specimen using a 5-ml sterile plastic syringe with a blunt-ended needle, by

slowly drawing water from the inhalant stream at a distance of $\sim$ 3 cm from the sponge ostia and then from the exhalant water inside the oscular aperture; care was taken to avoid contact with the sponge and one syringe was used per specimen. Once back in the laboratory, water samples were transferred into sterile 1.5 ml cryovials with freshly prepared glutaraldehyde (0.1% final concentration in distilled water). Samples were frozen in liquid nitrogen and stored at -80 °C following the protocol described by Marie et al. (1999) for natural seawater samples, until the flow cytometric analysis could be performed.

Seawater samples were analysed for quantification of heterotrophic bacteria and cyanobacteria (*Prochlorococcus* and *Synechococcus*) using a BD LSR II SORP (Special Order Research Product) cytometer equipped with five lasers. Identification of all organisms of interest was initially based on the DNA gate (see Chapter 2 for a detailed description of the flow cytometric method).

3.2.2 Data analysis

The retention efficiency was expressed as the percentage of picoplanktonic cells removed by each species from inhalant water samples and calculated as Equation 2.1. A two-way analysis of variance (ANOVA) was used to model the retention efficiency of sponges against sponge species (7 levels: *Dysidea* sp., *Plakina* sp., *Polymastia* sp., *L. echinata*, *Haliclona* sp., *T. bergquistae*, *Leucetta* sp.) and picoplankton (three levels: heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*). Since retention efficiency was expressed as percentage data, the data were arcsine-square root transformed to meet assumptions of normality and equal variance. The assumption of homogeneity of variances was examined using Bartlett's test ($P > 0.05$ in all cases).

Using data on the percentage retention (arcsine-square root transformed) of the three picoplankton types, the similarity in the diet composition of the seven sponge species was quantified using the Bray-Curtis dissimilarity index (Bray & Curtis 1957). From the resulting dissimilarity matrix, a dendrogram was constructed to visually display (in two dimensions) sponge species relationships, where species with more similar diet compositions were more

closely grouped. The dendrogram was constructed based on a Hierarchical cluster analysis. The idea behind a hierarchical cluster analysis is to show which of a set of samples are most similar to one another, and to group these similar samples in the same limb of a tree. Groups of samples that are distinctly different are placed in other limbs (Crawley, 2007). All statistical analyses were performed by R ver. 2.10 (R Development 6 Core Team 2011). The Vegan package was used to calculate the Bray-Curtis dissimilarity matrix and construct dendrograms.

3.3 Results

3.3.1 Picoplankton removal

Sponges removed three types of picoplanktonic organisms (heterotrophic bacteria–HetBact, *Prochlorococcus*–Prochlo, and *Synechococcus*–Synecho) from the ambient (inhalant) water. The overall average (± SD) ambient cell concentration of heterotrophic bacteria was markedly higher ($6.0 \times 10^5 \pm 2.4 \times 10^5$ cells ml^{-1}) than that of *Prochlorococcus* ($7.2 \times 10^4 \pm 5.2 \times 10^4$ cells ml^{-1}) and *Synechococcus* ($2.1 \times 10^4 \pm 1.4 \times 10^4$ cells ml^{-1}) for all samples taken from the inhalant water surrounding the study species. The retention efficiencies of the different picoplanktonic organisms varied considerably between the sponge species. There was a significant interaction in the retention efficiencies between species and types of picoplankton (ANOVA $F_{12,42} = 15.934$, $P < 0.001$) (Table 3.1). All species removed *Prochlorococcus* cells with the highest efficiency (range $53 - 94\%$), except for *L. echinata* which removed *Synechococcus* cells with the highest efficiency (71%). The retention efficiencies of heterotrophic bacteria and *Synechococcus* cells varied considerably between many of the species; heterotrophic bacterial cells were generally retained with the lowest efficiency (approximately 40%), Fig. 3.1.

3.3.2 Food resource utilisation

Results from the Bray-Curtis analysis which examined similarities in diet composition between the seven sponge species, showed that all species had a high similarity (dissimilarity range $0.22 - 0.33$; Table 3.2). However, the

Table 3.1: Two-way analysis of variance showing the interaction between sponge species and types of picoplankton. Significance at the 5% level

Source of variation	df	*F value*	*P value*
Species	6,42	33.154	$<$ **0.001**
Picoplankton	2,42	73.136	$<$ **0.001**
Species * Picoplankton	12,42	15.934	$<$ **0.001**

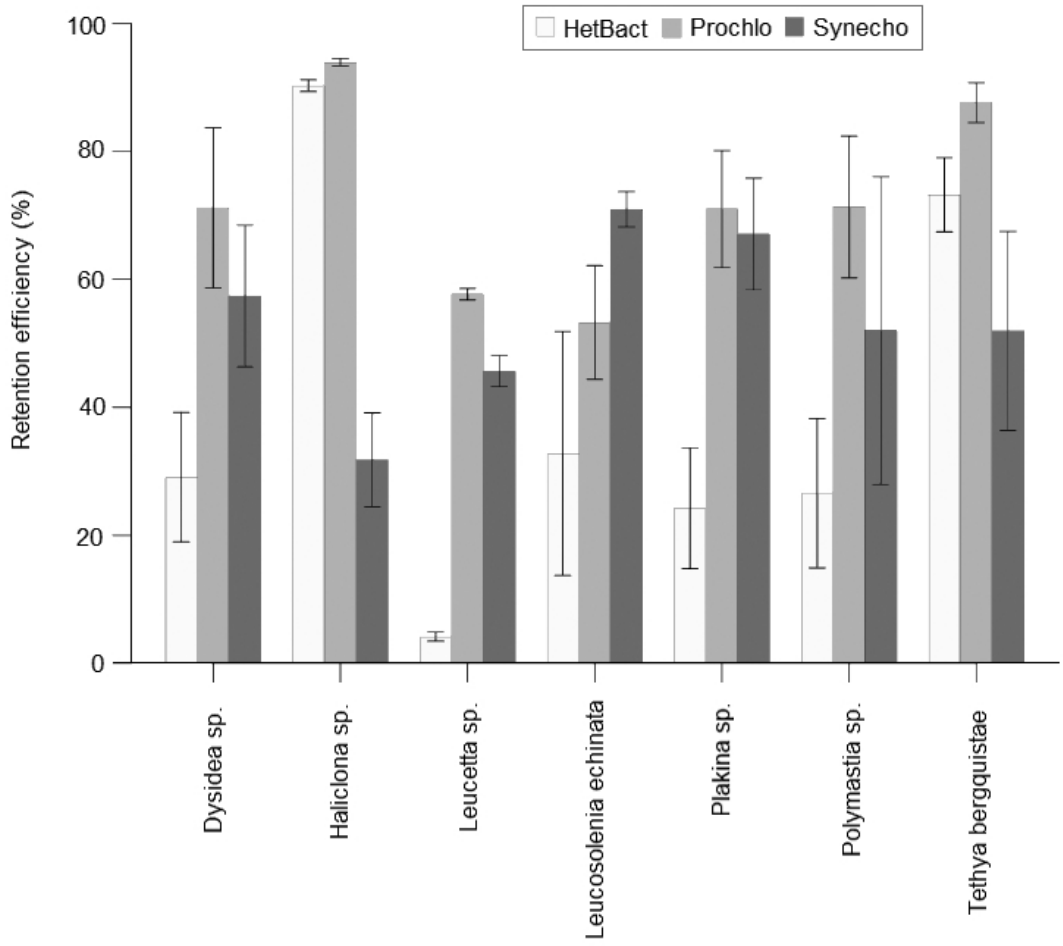


Figure 3.1: Retention efficiency (expressed as a percentage) of heterotrophic bacteria (HetBAct), *Prochlorococcus* (Prochlo), and *Synechococcus* (Synecho) cells removed by the study species as determined by two-way ANOVA.

Table 3.2: Bray-Curtis dissimilarity matrix of similarity in the diet composition of seven sponge species. Significance at the 5% level.

	Dysidea	*Haliclona*	*Leucetta*	*Leucosolenia*	*Plakina*	*Polymastia*
Haliclona	0.223					
Leucetta	0.151	0.333				
Leucosolenia	0.082	0.299	0.149			
Plakina	0.036	0.254	0.156	0.052		
Polymastia	0.022	0.223	0.129	0.089	0.042	
Tethya	0.128	0.087	0.257	0.204	0.16	0.132

dendrogram identified three groups of sponge species that retained similar amounts of the different picoplanktonic organisms: I) *Dysidea* sp., *Plakina* sp., *Polymastia* sp. and *L. echinata*; II) *Haliclona* sp. and *T. bergquistae*; III) *Leucetta* sp. (Fig. 3.2). Species in group I had moderate retention rates of *Prochlorococcus* cells ($53-71\%$), a retention of $52-67\%$ of *Synechococcus* cells, and a low retention rate of heterotrophic bacteria ($24-33\%$), while *L. echinata* was the only species that retained *Synechococcus* cells with a high efficiency (71%). Species in group II had high retention of *Prochlorococcus* cells ($88-94\%$) and heterotrophic bacteria ($73-90\%$), and removed less *Synechococcus* cells ($32-52\%$) than did sponges in group I. *Leucetta* sp. did not group with any of the other species and removed considerably more *Synechococcus* (46%) and *Prochlorococcus* cells (58%) than heterotrophic bacteria. Indeed, it was the only species that had a very low retention efficiency of heterotrophic bacteria (4%, Fig. 3.1).

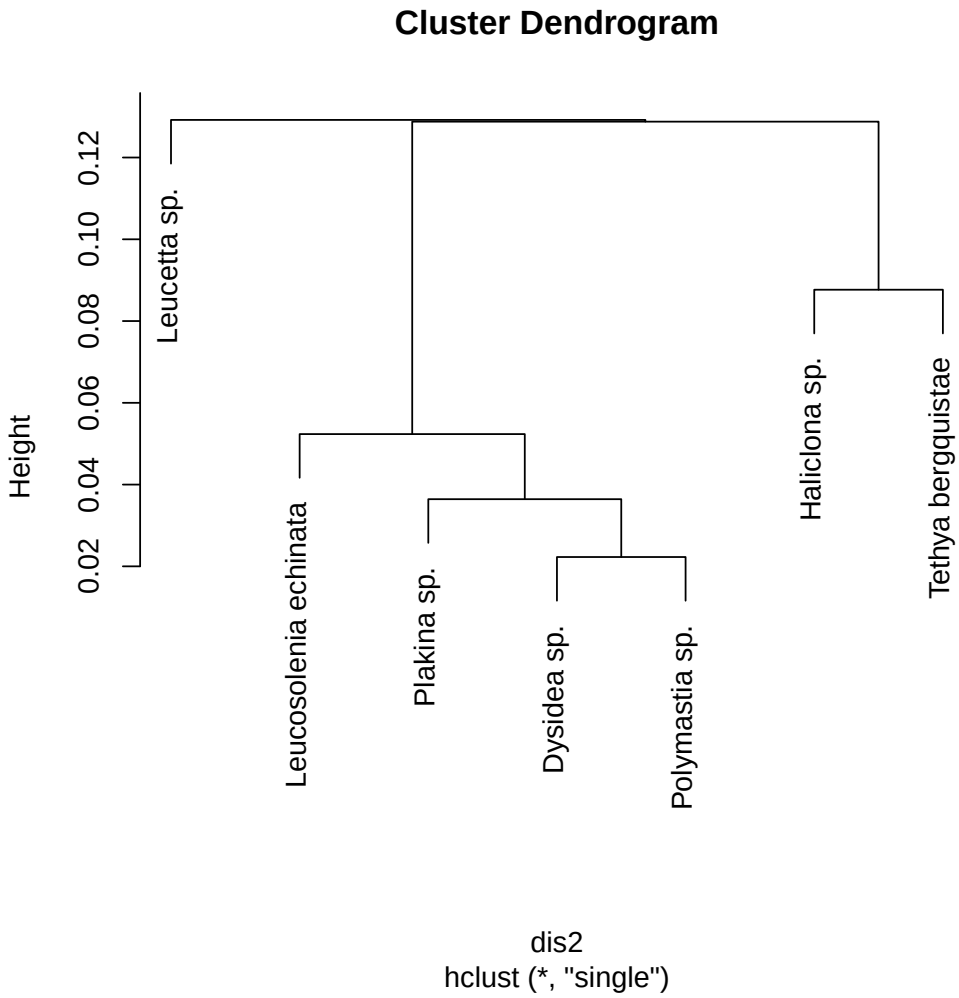


Figure 3.2: Cluster dendrogram (using Bray Curtis dissimilarity analysis) based on average retention values of the three different sponge dietary components. The analysis of variation between the study species yielded three groups: I) *Dysidea* sp., *Plakina* sp., *Polymastia* sp. and *Leucosolenia echinata*; II) *Haliclona* sp. and *Tethya bergquistae*; III) *Leucetta* sp. Species are more similar when dissimilarity is closer to zero.

3.4 Discussion

Suspension-feeders have developed specialisations to maximise prey capture that are directly linked to their survival. These include the spatial distribution of community members and the partitioning of available food resources by removing food particles in specific size ranges to avoid high levels of competition (Hartman 1957; Gili & Coma 1998). In some cases where species are able to utilise overlapping size fractions of particulate material, spatial separation can be an important factor in reducing competition between species (Stuart & Klumpp 1984). The aim of this study was to investigate if there was a differential particle size utilisation among different co-existing sponge species in the same habitat by examining variation in the food types that are consumed by sponges on a temperate rocky reef. The results demonstrated that these species are highly efficient at retaining cyanobacterial cells but with differing efficiencies according to cell type, indicating differences in resource use among the study species. Some of the species examined may be more efficient than others at exploiting different components of the available food in their environment, and I propose that differential resource use is occurring among the sponge species studied here based on the available food resources.

3.4.1 Is resource partitioning occurring based on preferences for different particle sizes?

Some authors have suggested that the retention mechanisms through which sponges differentiate between food particles are mainly based on particle size (Stuart & Klumpp 1984; Ribes et al. 1999a); however, there are researchers that have presented evidence suggesting that sponges do not select their food based upon particle size alone (see Osinga et al. 2001; Duckworth et al. 2006; Yahel et al. 2006). In the current study, different retention efficiencies were observed among the seven study species for the three types of picoplankton. The low retention efficiencies of heterotrophic bacteria by species in group I (*Dysidea* sp., *Plakina* sp., *Polymastia* sp. and *L. echinata*) suggest a preference by this group for larger cells (*Prochlorococcus* and *Synechococcus*); while species in group II (*Haliclona* sp. and *T. bergquistae*)

showed a higher retention of *Prochlorococcus* cells and heterotrophic bacteria, which are smaller than *Synechococcus* cells. The marked difference in the retention of *Synechococcus* and *Prochlorococcus* over heterotrophic bacteria by *Leucetta* sp. also implies a preference for bigger cells. I propose that in the temperate sponges studied here, particle size is an important factor influencing the retention of food particles given that five of the sponges retained bigger cells with a high efficiency and two sponge species (*Haliclona* sp. and *T. bergquistae*) had high retention of smaller cells (heterotrophic bacteria). However, particle size may not be the single defining factor as suggested by Yahel et al (2006) in selecting food particles, but instead it may depend on each individual sponge species; this is supported by previous studies where some sponges have low efficiency for smaller particles (e.g. Kowalke 2000), and others retain the smallest prey types with high efficiency (e.g. Ribes et al. 1999a).

3.4.2 Ambient cell concentration

Previous work has demonstrated that the retention efficiency of sponges depends on the bacterial concentration in the surrounding water (Duckworth et al. 2003). Experimental studies on sponges have concluded that low cell concentrations favour higher retention efficiencies, while high concentrations result in decreased retention efficiencies by sponges (Huysecom et al. 1988; Duckworth et al. 2003). The previously observed decreases in retention efficiency in the presence of high picoplanktonic concentrations have been attributed to apparent blocking of the aquiferous system or a complete contraction of the sponge (Huysecom et al. 1988). Interestingly, the ambient cell concentrations showed that heterotrophic bacteria were the most abundant cells found in the inhalant/ambient water, although only species in group II retained these cells with a high efficiency ($73 - 90\%$). High concentrations of heterotrophic bacteria were also measured in the ambient water of species in group I, but these species had the lowest retention of these picoplanktonic cells ($24 - 33\%$). In contrast, *Prochlorococcus* were far less abundant than heterotrophic bacteria, and species in group II had high retention rates of these cells ($88 - 94\%$) supporting the hypothesis that lower concentrations of

cells favour higher efficiency (Huysecom et al. 1988; Duckworth et al. 2003). However, it is important to note that this pattern was only found in this group. *Synechococcus* were the least abundant cell type in the ambient water and retention efficiencies were highly variable between species ($32 - 71\%$). Because there was no consistent pattern for all species, I suggest that the variability in retention efficiency for the species studied here is not directly influenced by the food concentration in the water column, as has been suggested by previous studies (Huysecom et al. 1988; Duckworth et al. 2003). However, this issue will be discussed in more detail in Chapters 4 and 6.

3.4.3 Food availability

In view of the fluctuations in food availability that suspension-feeders are likely to experience, seasonal fluctuations in the abundance of planktonic organisms are expected in temperate regions; at times there will be a higher abundance of available food, while at other times the amount of food will be limited (Hartman 1957). In Chapter 6, I examine the seasonal and interannual fluctuations in the abundance and relative proportion of the picoplanktonic species composition available in the water column. For active suspension-feeders, both food concentration and flow speed are likely to influence food availability (Leichter & Witman 1997; Ribes et al. 1999a); furthermore, the availability of picoplanktonic organisms may vary temporally and spatially. The cell concentrations showed a consistent or homogeneous concentration of picoplanktonic cells in the ambient (inhalant) water throughout the sampling period, though I did only sample at one time of year within a single year. Consequently, in the sampling period, the cell (i.e. food) concentration in the ambient water did not appear to account for differences in the retention efficiencies observed. However, analysis of temporal (i.e. seasonal) and possibly diel patterns of food capture may also play an important role in the partitioning of resources, since the availability of picoplanktonic organisms is expected to change at these scales (Porter 1976; Yahel et al. 2003); this will be addressed and discussed in Chapter 6.

3.4.4 Nutritional value of the particles retained

The variability in retention efficiency between sponge species might be a result of differences in the nutritional value of the picoplanktonic cell types and the specific nutritional requirements of each sponge species. Previous studies have suggested that the carbon requirements of temperate sponges can be met by the utilisation of food in the size fraction $< 1 - 5$ µm (Stuart & Klumpp 1984; Ribes et al. 1999b). For example, during periods of high bacterial numbers, free-living bacteria were found to provide 17% of the carbon requirements in the temperate encrusting demosponge *Haliclona anonyma* (Stuart & Klumpp 1984), and in a study of two temperate sponge species prokaryotes comprised 74% of the total ingested carbon (Ribes et al. 1999a). Accordingly, the three populations of picoplanktonic organisms identified in this study are prokaryotes within the $0.8 - 1$ µm size range, and are well-known components of the natural diet of temperate demosponges (Pile et al. 1996; Ribes et al. 1999a; Pile 2005); though *Prochlorococcus* were not identified in a recent study of temperate sponges in Australia (Hanson et al. 2009). Sponge species in group I were less efficient at removing the smallest and most abundant of the bacterial organisms (heterotrophic bacteria). In contrast, species in group II efficiently removed up to 94% of the *Prochlorococcus* cells and up to 90% of heterotrophic bacteria, while *Leucetta* sp. had low retention efficiency of heterotrophic bacteria (4%). The differences in retention of the three cell types suggest that the study species prefer some food particles over others. It remains to be examined if the fewer larger cells (i.e., *Prochlorococcus* and *Synechococcus*) are able to provide the same amount of energy required to meet the sponges' metabolic demands as are smaller, more abundant cells (heterotrophic bacteria), when retained with a high efficiency.

The physiological condition of the sponges might also play a role in the different retention efficiencies observed. Huysecom et al. (1988) suggested that the nutritional status of a species may influence retention rates, while Osinga et al. (2001) assumed that the differences in retention efficiencies for a tropical sponge were caused by the physiological condition of the sponge. As mentioned above, bacteria and other small particles in the water column

are an important food resource for sponges, but sponges also support diverse symbiotic assemblages (Taylor et al. 2007). Hence, some retention efficiency might reflect the degree of reliance on additional nutrition from photosynthetic micro-symbionts versus exogenous food. There is currently no information on the symbiotic associations of the sponge species studied here. However, there are studies that have determined the presence of photosynthetic symbionts (cyanobacteria) in other temperate species of the genus *Dysidea* sp. (Ribes et al. 1999a), *Tethya* sp. (Thiel et al. 2007) and *Crella* sp. (Lemloh et al. 2009); and from one species of *Leucetta* sp. from the Great Barrier Reef in Australia (Kehraus et al. 2002). The potential effects of symbiotic bacteria on the nutrition of these different sponge species needs to be elucidated to better understand their needs for exogenous food and may provide further evidence to support niche partitioning in these species based on food particle size preferences and nutritional demands.

Particle size is thought to play an important role in the partitioning of food resources between co-existing suspension-feeders. This is supported by my study, which suggests that the size of particles influences retention efficiency and the preference of food particles when examining several co-existing sponge species sharing the same habitat. The results presented here indicate clear differences in resource use among the study species, suggesting that some of the species examined may be more efficient at exploiting different components of the available food resource in their environment than are others. I propose a number of possible factors that can help understand these differences (e.g. particle size, ambient cell concentration, nutritional value of the particles retained). However, it is still unclear which factor has the greatest influence as different factors seem to influence the retention efficiency of each species. There has been much debate as to whether greater resource extraction among diverse communities results from resource partitioning, or rather from the greater likelihood of including particularly efficient species at higher diversity levels (Northfield et al. 2010). Similarly, if there is significant dietary differentiation between co-existing species, a more detailed analysis is needed to clarify whether it is linked to strict partitioning of distinct resources or to different relative consumption of shared resources (Martin & Genner 2009). This study is the first to examine resource util-

isation of sponges *in situ* within a temperate rocky reef where many species co-exist with apparently similar resource requirements. In the present study food-use relationships were examined, but did not evaluate competitive relationships between the study species. However, the results presented here provide a potential mechanism for explaining the high diversity of sponges in a particular habitat where they are apparently sharing the same food resource, with different species focusing on different groups of picoplanktonic organisms. This plasticity in trophic ecology may be one of the main factors contributing to the worldwide success of sponges despite large spatial and temporal variation in food sources (Ribes et al. 1999a).

To conclude, the findings from this study support the partitioning of food resources between different co-existing sponge species, where some similarities were observed in the retention of cells among groups of species; however, the seven study species had different feeding preferences. I propose that the ability of different species to focus on different picoplanktonic organisms with varying efficiencies represents differential particle size utilisation and selective utilisation of resources, so allowing co-existence of different sponge species in the same habitat. While the data presented here suggest that niche partitioning can explain the varying efficiencies between species, it also suggests that partitioning may not be essential for co-existence, as some species had similar retention efficiencies implying an overlap in resource use.

3.5 References

— Bastolla U., Lassig M., Manrubia S.C. & Valleriani A. (2005). Biodiversity in model ecosystems, I: coexistence conditions for competing species. Journal of Theoretical Biology, 235, 521-530.

— Bell J.J. (2008). The functional roles of marine sponges. Estuarine, Coastal and Shelf Science, 79, 341-353.

— Berman J. & Bell J. (2010). Spatial Variability of Sponge Assemblages on the Wellington South Coast, New Zealand. Open Marine Biology Journal, 4, 12-25.

— Berman J., Perea-Blázquez A., Kelly M. & Bell J. (2008). Sponges of the Wellington south coast. In: The Taputeranga Marine Reserve (eds. Gardner J & Bell J). First Edition Ltd Wellington, pp. 225-236.

— Bray J.R. & Curtis J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs, 27, 325-349.

— Crawley M.J. (2007). The R book. John Wiley & Sons Ltd, London UK.

— Darwin C. (1859). The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. 1958 ed. Mentor, New York.

— Duckworth A.R., Bruck W.M., Janda K.E., Pitts T.P. & McCarthy P.J. (2006). Retention efficiencies of the coral reef sponges *Aplysina lacunosa*, *Callyspongia vaginalis* and *Niphates digitalis* determined by Coulter counter and plate culture analysis. Marine Biology Research, 2, 243-248.

— Duckworth A.R., Samples G.A., Wright A.E. & Pomponi S.A. (2003). In vitro culture of the tropical sponge *Axinella corrugata* (Demospongiae): Effect of food cell concentration on growth, clearance rate, and biosynthesis of stevensine. Marine Biotechnology, 5, 519-527.

— Flint R.W. & Kalke R.D. (1986). Niche characterization of dominant estuarine benthic species. Estuarine Coastal and Shelf Science, 22, 657-674.

— Genner M.J., Turner G.F. & Hawkins S.J. (1999). Foraging of rocky habitat cichlid fishes in Lake Malawi: coexistence through niche partitioning? Oecologia, 121, 283-292.

— Gili J.M. & Coma R. (1998). Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution, 13, 316-321.

— Hanson C.E., McLaughlin M.J., Hyndes G.A. & Strzelecki J. (2009). Selective uptake of prokaryotic picoplankton by a marine sponge (*Callyspongia* sp.) within an oligotrophic coastal system. Estuarine Coastal and Shelf Science, 84, 289-297.

— Hartman W.D. (1957). Ecological niche differentiation in the boring sponges (Clionidae). Evolution, 11, 294-297.

— Hentschel U., Usher K.M. & Taylor M.W. (2006). Marine sponges as microbial fermenters. FEMS Microbiology Ecology, 55, 167-177.

— Hines A.H. (1982). Coexistence in a kelp forest: size, population dynamics and resource partitioning in a guild of spider crabs (Brachyura, Majidae). Ecological Monographs, 52, 179-198.

— Huysecom J., Richelle-Maurer E., Vyver G.V.D. & Vray B. (1988). Effect of bacterial concentration on retention and growth rate of the freshwater sponge *Ephydatia fluviatilis*. Physiological Zoology, 61, 535-542.

— Kehraus S., Konig G.M., Wright A.D. & Woerheide G. (2002). Leucamide A: A new cytotoxic heptapeptide from the Australian sponge *Leucetta microraphis*. Journal of Organic Chemistry, 67, 4989-4992.

— Kowalke J. (2000). Ecology and energetics of two Antarctic sponges. Journal of Experimental Marine Biology and Ecology, 247, 85-97.

— Leibold M.A. (1995). The niche concept revisited: Mechanistic models and community context. Ecology, 76, 1371-1382.

— Leichter J.J. & Witman J.D. (1997). Water flow over subtidal rock walls: Relation to distributions and growth rates of sessile suspension feeders in the Gulf of Maine - Water flow and growth rates. Journal of Experimental Marine Biology and Ecology, 209, 293-307.

— Lemloh M.-L., Fromont J., Brummer F. & Usher K.M. (2009). Diversity and abundance of photosynthetic sponges in temperate Western Australia. BMC Ecol, 9, 4.

— Marie D., Brussaard C.P.D., Thyrhaug R., Bratbak G. & Vaulot D. (1999). Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied Environmental Microbiology, 65, 45-52.

— Martin C.H. & Genner M.J. (2009). High niche overlap between two successfully coexisting pairs of Lake Malawi cichlid fishes. Canadian Journal of Fisheries and Aquatic Sciences, 66, 579-588.

— Menge B.A., Daley B.A., Wheeler P.A., Dahlhoff E., Sanford E. & Strub P.T. (1997). Benthic-pelagic links and rocky intertidal communities: Bottom-up effects on top-down control? Proceedings of the National Academy of Sciences, 94, 14530-14535.

— Northfield T.D., Snyder G.B., Ives A.R. & Snyder W.E. (2010). Niche saturation reveals resource partitioning among consumers. Ecology Letters, 13, 338-348.

— Orejas C., Gili J.M., Arntz W.E., Ros J.D., Lopez P.J., Teixido N. & Filipe P. (2000). Benthic suspension feeders, key players in Antarctic marine ecosystems? Contributions in Science, 1, 299-311.

— Osinga R., Kleijn R., Groenendijk E., Niesink P., Tramper J. & Wijffels R.H. (2001). Development of *In vivo* sponge cultures: particle feeding by the tropical sponge *Pseudosuberites* aff. *andrewsi*. Marine Biotechnology, 3, 544-554.

— Patten B.C. & Auble G.T. (1981). System Theory of the Ecological Niche. The American Naturalist, 117, 893-922.

— Pile A.J. (2005). Overlap in diet between co-occurring active suspension feeders on tropical and temperate reefs. Bulletin of Marine Science, 76, 743-749.

— Pile A.J., Patterson M.R. & Witman J.D. (1996). *In situ* grazing on plankton <10 um by the boreal sponge *Mycale lingua*. Marine Ecology Progress Series, 141, 95-102.

— Pile A.J. & Young C.M. (2006). The natural diet of a hexactinellid sponge: Benthic-pelagic coupling in a deep-sea microbial food web. Deep-Sea Research Part I-Oceanographic Research Papers, 53, 1148-1156.

— Porter J.W. (1976). Autotrophy, heterotrophy, and resource partitioning in Caribbean reef-building corals. American Naturalist, 110, 731-742.

— R Development Core Team, 2011. R: A Language and Environment for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

— Reiswig H.M. (1971b). Particle feeding in natural population of 3 marine demosponges. Biological Bulletin, 141, 568-591.

— Reiswig H.M. (1973). Population dynamics of three Jamaican Demospongiae. Bulletin of Marine Science, 23, 191-226.

— Reiswig H.M. (1974). Water transport respiration and energetics of 3 tropical marine sponges. Journal of Experimental Marine Biology and Ecology, 14, 231-249.

— Ribes M., Coma R., Atkinson M.J. & Kinzie R.A. (2005). Sponges and ascidians control removal of particulate organic nitrogen from coral reef water. Limnology and Oceanography, 50, 1480-1489.

— Ribes M., Coma R. & Gili J.M. (1999a). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Ribes M., Coma R. & Gill J.M. (1999b). Seasonal variation of particulate organic carbon, dissolved organic carbon and the contribution of microbial communities to the live particulate organic carbon in a shallow near-bottom ecosystem at the Northwestern Mediterranean Sea. Journal of Plankton Research, 21, 1077-1100.

— Ross S.T. (1986). Resource partitioning in fish assemblages: A review of field studies. Copeia, 352-388.

— Sala E. & Ballesteros E. (1997). Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem. Marine Ecology Progress Series, 152, 273-283.

— Shmida A. & Ellner S. (1984). Coexistence of plan species with similar niches. Vegetatio, 58, 29-55.

— Stuart V. & Klumpp D.W. (1984). Evidence for food-resource partitioning by kelp-bed filter feeders. Marine Ecology Progress Series, 16, 27-37.

— Taylor M.W., Radax R., Steger D. & Wagner M. (2007). Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiology and Molecular Biology Reviews, 71, 295-347.

— Thiel V., Neulinger S.C., Staufenberger T., Schmaljohann R. & Imhoff J.F. (2007). Spatial distribution of sponge-associated bacteria in the Mediterranean sponge *Tethya aurantium*. FEMS Microbiology Ecology, 59, 47-63.

— Thurber A.R. (2007). Diets of Antarctic sponges: links between the pelagic microbial loop and benthic metazoan food web. Marine Ecology-Progress Series, 351, 77-89.

— Tilman D. (2004). Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. Proceedings of the National Academy of Sciences, 101, 10854-10861.

— Turon X., Galera J. & Uriz M.J. (1997). Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. Journal of Experimental Zoology, 278, 22-36.

— Witte U., Brattegard T., Graf G. & Springer B. (1997). Particle capture and deposition by deep sea sponges from the Norwegian-Greenland Sea. Marine Ecology Progress Series, 154, 241-252.

— Wulff J.L. (2006). Ecological interactions of marine sponges. Canadian Journal of Zoology, 84, 146-166.

— Yahel G., Eerkes-Medrano D.I. & Leys S.P. (2006). Size independent selective filtration of ultraplankton by hexactinellid glass sponges. Aquatic Microbial Ecology, 45, 181-194.

— Yahel G., Sharp J.H., Marie D., Hase C. & Genin A. (2003). *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography, 48, 141-149.

— Yahel G., Whitney F., Reiswig H.M., Eerkes-Medrano D.I. & Leys S.P. (2007). *In situ* feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. Limnology and Oceanography, 52, 428-440.

Chapter 4

Energy flow from the pelagic environment to the benthos through sponge assemblages

Abstract

Trophic interactions between organisms are important drivers of marine ecosystem dynamics, particularly because they are the primary means by which solar energy is transferred along food chains, from primary producers to top predators. Despite the importance of trophic interactions, and the relationship between primary production and benthic diversity, there have been few studies that have quantified the energy flow from pelagic to benthic environments at the assemblage level as a result of the activities of suspension-feeding organisms. Seven of the most common sponge species from the Taputeranga marine reserve, located on the south coast of Wellington, were selected for this study. A number of characteristics of these sponges were analysed, including diet composition, feeding efficiency, pumping rates, and the number of food particles (prokaryotic cells) retained. I used this information, combined with abundance estimates of the sponges and estimations of the total amount of food available to sponges in a known volume of water (89,821 m^3), to estimate: (1) carbon fluxes, used as an indicator of the energy flux through sponges, as a result of their suspension-feeding activities; and (2) the proportion of the available carbon that they consume. Most of the carbon gained by the sponges was from heterotrophic bacterial cells (range of 1.9 to 95.6 g C d^{-1}) with varying sponge percentage cover (from 0.5 to 5% cover), followed by *Prochlorococcus* (1.7 to 83.6 g C d^{-1}) and then *Synechococcus* (1.0 to 48.9 g C d^{-1}), which corresponds with the rank order of abundance of each of the picoplanktonic organisms in the surrounding water. Depending on sponge abundance (estimates ranging from 0.5 to 5% cover), the amount of C that sponges consumed as a pro-

portion of the total POC available was $0.2 - 12.1\%$ for heterotrophic bacteria, $0.4 - 21.3\%$ for *Prochlorococcus*, and $0.3 - 15.8\%$ for *Synechococcus*. The flux of carbon for the whole sponge assemblage, based on the consumption of prokaryotic picoplankton, ranged from 70 to 3,500 mg C m^2 d^{-1}. The results presented here show that sponge assemblages on subtidal rocky reefs are capable of consuming a considerable proportion of the available picoplankton, and are therefore responsible for the transfer of large amounts of carbon from the water column to the benthos. This study is the first to estimate the contribution of a sponge assemblage (rather than focusing on individual sponge species) to carbon flow in a temperate rocky reef through the sponges feeding activity and demonstrates the importance of sponges to energy flow in rocky reef environments.

4.1 Introduction

In biological systems, many species interact through complex trophic networks where bottom-up and top-down controls continuously interact (e.g. Menge et al. 1997; Menge 2000). In these networks or food webs, an organism might be directly transferring energy via only a few other species, but these species are likely linked, both directly and indirectly, with numerous other species (Szyrmer & Ulanowicz 1987). These linkages are the main drivers of marine ecosystem dynamics, as they transfer and dissipate solar energy from primary producers to top predators (Maury et al. 2007). The trophic relationships between benthic and pelagic communities mainly depend on the movement of primary production in surface waters to deeper layers (Gili et al. 2006). In highly productive marine areas, the major biological factors structuring benthic communities are recruitment and the flow of organic matter from the pelagic domain to the benthos (Cattaneo-Vietti et al. 1999). High water motion in coastal zones increases the flow of nutrients between the pelagic and benthic environments making the study of benthic trophodynamics (i.e. the flow of energy and particles) important for understanding the dynamics of coastal systems (Lopez et al. 2006). Furthermore, benthic marine food webs are essential biological components of coastal ecosystems because of their role in organic matter cycling and because they provide a link between the water column, benthic organisms and sediments (Herman et al. 1999).

Suspension-feeding is one of the most widespread feeding strategies among benthic organisms including members of the Porifera, Cnidaria, Bryozoa,

Brachiopoda, Annelida (Polychaeta), Mollusca (Bivalvia), Echinodermata, Crustacea and Tunicata (Coma et al. 2001). Suspension-feeding invertebrates play an important role in the flow of carbon through marine ecosystems as they have the ability to control the cycling of nutrients, organic matter, plankton and detritus (Stuart & Klumpp 1984; Gili & Coma 1998; Ribes et al. 2005), and move carbon from the pelagic environment to the benthos (and vice versa). Benthic-suspension feeders are considered among the most efficient organisms at extracting and processing energy from marine ecosystems (Gili & Coma 1998) and the trophic strategies of these organisms are strongly related to the availability of carbon occurring in the water column (Cattaneo-Vietti et al. 1999). Hence, studying the feeding ecology of these organisms is important for understanding the dynamics of particles in the water column and energy flow in marine ecosystems. Sponges are one of the most important components of the suspension-feeding community in rocky environments, as they are very abundant and are able to effectively exploit pelagic food resources, thus providing coupling between primary production and the benthos by converting planktonic carbon into sponge biomass (Pile et al. 1997; Trussell et al. 2006). This carbon can then be used by higher trophic levels through the consumption of sponge biomass by organisms such as fish, sea stars (Loh & Pawlik 2009), turtles (Meylan 1988; Leon & Bjorndal 2002), sea urchins (Ayling 1981; Wright et al. 1997) and opisthobranchs (Becerro et al. 2003). Alternatively, sponges may act as a carbon sink, since many species are long-lived and unpalatable to potential predators (Pile & Young 2006; Peters et al. 2009).

Both photoautotrophic and heterotrophic picoplankton are important components of global marine primary production (Partensky et al. 1999; Ting et al. 2002) since they are major participants in global carbon cycles (Ting et al. 2002). Photoautotrophic picoplankton (< 2 µm in size) are single-celled free-living cyanobacteria in the water column dominated by two genera; *Prochlorococcus* and *Synechococcus* (Waterbury et al. 1979; Chisholm et al. 1992). These organisms occupy key positions at the base of marine food webs, and their abundance and productivity potentially dictate the flow of carbon through food webs (Scanlan 2001). The carbon sequestered as a result of photosynthesis is moved to higher trophic levels via intermediate

small grazers, such as flagellates (Guillou et al. 2001) and ciliates (Christaki et al. 1999), which are most likely major consumers of *Prochlorococcus* and *Synechococcus*; this represents a trophic link between picoplankton primary producers and higher trophic levels (Christaki et al. 1999). *Prochlorococcus* and *Synechococcus* cells are too small to be consumed directly by other components of the plankton, such as small copepods and cladocerans. However, they are a significant food resource for larger benthic suspension-feeding organisms such as bivalves, ascidians and sponges (Langdon & Newell 1990; Gili & Coma 1998; Ribes et al. 2005; Yahel et al. 2005).

Previous research has demonstrated that sponges efficiently feed on *Prochlorococcus*, *Synechococcus* and heterotrophic bacterial cells, and are capable of moving large quantities of these organisms from the pelagic environment to the benthos (Pile 1997; Ribes et al. 1999a). In addition, species-level studies of plankton removal by sponges and their role in bottom-up effects (Trussell et al. 2006; Hanson et al. 2009) have shown that sponges are significant sinks for particulate organic material (POM) and for dissolved organic carbon (DOC) (Yahel et al. 2003); and recently, a study has provided direct evidence for the incorporation of dissolved organic matter (DOM) by sponges (de Goeij et al. 2008). Previous studies have examined the natural diet of temperate demosponges using different *in situ* techniques. However, these have only been conducted on a small number of species (Ribes et al. 1999a; Pile 2005; Yahel et al. 2005; Hanson et al. 2009) and the ecosystem-level effects of sponge feeding have not yet been estimated.

Recent reviews on the functional roles that sponges play in marine systems (Wulff 2006; Bell 2008) have highlighted the ecological importance of sponges, particularly in habitats where they occur in high densities. Despite their potentially important interaction with the water column, many aspects of sponge biology and ecology remain poorly described, and as a result our knowledge of the energy transfer from pelagic to benthic habitats resulting from feeding by sponge assemblages remains poor. This study determined the ecological importance of a temperate sponge assemblage with respect to its contribution to energy flow from the water column to the benthos, by measuring rates of carbon consumption in the form of particulate matter (food particles). This information was combined with data on the charac-

teristics of the sponge species (diet composition, sponge abundance, feeding efficiency, pumping rate and number of food particles removed), to estimate the proportion of the available carbon in the water column of a defined coastal region that was being consumed by the sponge assemblage.

4.2 Methods

4.2.1 Study site and in situ *sampling*

The study sites are located within the Taputeranga Marine Reserve on the south coast of Wellington in New Zealand (see Chapter 2 for more details). Seven of the most common and widespread sponge species from the area were selected for this work: *Dysidea* sp., *Haliclona* sp., *Plakina* sp., *Polymastia* sp., *Tethya bergquistae* (Hooper, 1994), *Leucetta* sp., and *Leucosolenia echinata* (Kirk, 1893)[1]. These species were chosen because they are very common in the study area and have well defined exhalant oscula that reduce the risk of sampling error, so facilitating *in situ* water sampling.

4.2.2 Collection of water samples

Seawater samples were collected *in situ* using SCUBA. Sampling was conducted at high tide. Samples were collected between November 2008 and January, February and March 2009. This sampling interval reflects the difficulty of sampling within this study area due to the very dynamic environment. The samples were randomly collected over this period to avoid any potential bias as a result of the length of the sampling. Three specimens of each species were randomly selected and fluorescein dye was released at the base of each specimen to visually confirm that sponges were actively pumping. Water samples were taken by using 5-ml sterile plastic syringes with blunt-ended needles. The inhalant water of each specimen was sampled by slowly drawing water at a distance of $\sim$ 3 cm from the sponge ostia, and the exhalant water was sampled from the inside oscular aperture taking care to avoid contact with the sponge. Each specimen was photographed *in situ* next to a ruler to measure the height, width and length of each sponge, to

[1] see species photos in Appendix A on page 155

relate areal cover to sponge biomass. The number and diameter of all oscula per sponge specimen were recorded and measured with the ruler. After collection, water samples were transferred into sterile 1.5 ml cryovials with freshly prepared glutaraldehyde (0.1% final concentration), frozen in liquid nitrogen and stored at −80 °C following the protocol described by Marie et al. (1999a) for natural seawater samples, until the flow cytometric analysis could be performed.

4.2.3 Flow cytometry and data analyses

In preparation for flow cytometric analysis, samples were thawed to room temperature, then stained in the dark with the DNA-specific dye Hoechst 33342 (0.2 µg ml^{-1} final concentration) for bacterial identification. Seawater samples were analysed for quantification of heterotrophic bacterial and cyanobacterial (*Prochlorococcus* and *Synechococcus*) cells using a BD LSR II SORP (Special Order Research Product) cytometer equipped with five lasers (see Chapter 2 for a detailed description of the flow cytometric method).

Data for natural samples are typically collected for 2 to 4 minutes with a flow rate of 50 to 100 µl min^{-1} (Marie et al. 1999a). All samples were run at a high flow rate (100 µl min^{-1}) and the analysis time was recorded to precisely determine the cell concentrations of each type of picoplankton. The absolute cell concentrations for each population in a given sample were calculated as follows:

$$C_{pop} = \frac{T * N_{pop}}{R * (V_{total}/V_{sample})} \tag{4.1}$$

Where: C_{pop} is the concentration of picoplankton in cells μl^{-1}; N_{pop} is the number of cells acquired; T is the acquisition time (min); R is the sample flow rate in µl min^{-1}; V_{total} is the volume (µl) of sample plus additives (fixatives, dyes, beads, etc.); and V_{sample} is the volume of sample analysed on µl (Marie et al. 1999a).

4.2.4 Measurement of sponge pumping rates

Pumping rate estimations were performed during the sampling months through dye-release experiments. Sponges were filmed *in situ* and sodium fluorescein dye was released next to the sponge. The pumping activity of three specimens of each species was first visualised and recorded by releasing dye at the base of the specimen and observing the movement of the dye through the sponge. A ruler was placed next to the sponge specimen and used as a scale reference in the field of view of the camera. Subsequent frame-by-frame image and video analyses were performed to estimate pumping velocity, where only frames showing the vertical movement of the dye through 2 cm of water immediately above the osculum were used to measure the distance travelled by the dye-plume per unit time (Savarese et al. 1997). Volume flux or pumping rate (Q), which is the volume of water exiting an osculum per unit time, was calculated by multiplying the exhalant flow speed (v) expressed in cm s-1, by the cross-sectional area of the osculum (A), using the equation:

$$Q = vA \tag{4.2}$$

This calculation assumes plug flow which is most likely true for sponges (see Savarese et al. 1997). Volume flow rate (the total volume of water processed per unit time, s^{-1}) was then estimated by multiplying the pumping rate (Q) by the number of oscula per sponge, as the study species are all multi-oscular sponges (Savarese et al. 1997).

4.2.5 Retention efficiency and number of cells filtered

Retention efficiency, expressed as the percentage of picoplanktonic cells removed by three specimens of each species from inhalant water samples, was calculated as Equation 2.1. Then the number of cells filtered was calculated by multiplying: retention efficiency (no units), volume flow rate (ml s^{-1}) and ambient concentration of cells (cells ml^{-1}), as described by Trussell et al. (2006). All means are presented with standard deviations.

4.2.6 Carbon flux estimations

Estimates of particulate organic carbon (POC) from the picoplanktonic organisms were obtained using the mean number of cells removed per ml^{-1}, as determined by flow cytometric analysis. This value was then converted to mg of C for each type of picoplankton using the following standard cell conversions from the literature: heterotrophic bacteria, 20 fg C $cell^{-1}$ (Ducklow et al. 1993); *Prochlorococcus* sp., 61 fg C $cell^{-1}$ (Bertilsson et al. 2003); *Synechococcus* spp., 178 fg C $cell^{-1}$ (Charpy & Blanchot 1998). These conversions were used because they were calculated for cells with mean diameters that correspond to the cell diameters found during the present study (Pile 1997), which were visually confirmed using confocal microscopy. For each sponge specimen, carbon acquired per second was calculated by multiplying the number of cells retained (cells ml^{-1} s^{-1}) by the quantity of carbon contained in each type of cell (Trussell et al. 2006). The data are presented in such a way that POC fluxes can be re-calculated if more accurate carbon equivalents become available for the picoplanktonic organisms specific to the study area.

4.2.7 Sponge abundance calculations

The calculations above provide estimates of the carbon consumed by individual sponges per unit time; however the intention was also to calculate the amount of POC consumed by a sponge assemblage. For this purpose, the abundance of sponges, the volume of water in a known area (Taputeranga Marine Reserve) and the amount of POC contained within the water (based on the data from the ambient water), were estimated.

The Wellington south coast supports diverse sponge assemblages with up to 500 sponges per m^2 in some areas, covering over 50% of the substratum at some sites (Berman & Bell 2010). At the study sites, sponge percentage cover and sponge density have been previously estimated from 0.5 m^2 photoquadrats (Berman & Bell 2010) for the most abundant species, including the study species selected for this study. The results from these earlier surveys showed that sponge coverage is highly variable; therefore, a range of values for sponge percentage cover was used for all seven species combined. In order to account

for the high variability in the study region and because it was not possible to sample the entire reserve area, low, mid and high estimates (0.1, 0.5, 1, 1.5 and 5%) of sponge coverage were used based on the coverage calculated for the study species living on vertical rock walls. The different values of sponge coverage were used for subsequent calculations and all the characteristics (diet composition, sponge abundance, feeding efficiency, pumping rate and number of food particles removed) analysed from the seven study species were used as a representation of the sponge assemblage for the given range of sponge abundances. In order to integrate the information of the amount of POC consumed by individual sponges to estimate the amount of POC consumed by a sponge assemblage, calculations were made using the measured sponge areas of the study species and by assuming a uniform sponge thickness of 1 cm (which is based on field observations of the species).

To estimate the volume of water in the reserve, I compiled information on bathymetry and total rocky reef area from habitat maps of the study region (Wright et al. 2006). I binned the area into several regions and then calculated the total volume of water in m^3 based on the average depth for the region using the different depth ranges from the bathymetry maps. Similar calculations were performed using the submarine rocky area to estimate the total area of reef in the reserve expressed in km^2. To estimate the percentage of picoplanktonic POC removed by the sponges from the total available in the water column, we assumed a homogeneous distribution of bacterioplankton throughout the water column. In a coastal turbulent environment such as the one studied here, there is likely to be enough mixing by wave action to make the first 10 m or so homogenous. This has recently been confirmed by ongoing studies at Victoria University of Wellington in the same area, where chlorophyll records showed no variation between $0 - 10$ m depth (César A. Cárdenas personal communication).

4.2.8 Supporting calculations

Based on the calculated dimensions of the study area, and using the data from all seawater samples collected, the ambient number of picoplanktonic cells present in the volume of water in the study area at any one time was

calculated as:

$$\frac{ambCell * volSA}{equ} \tag{4.3}$$

Where $ambCell$ is the ambient concentration of cells (cells ml^{-1}), $volSA$ is the volume of water in the study area (m^3) and equ is the equivalent of 1 ml in m^3 (0.000001). Accordingly, the number of cells that sponge assemblages would be capable of removing on a daily basis in the study area (assuming that sponges were actively pumping for 24 hours per day) was estimated using a variation of the previous equation:

$$\frac{ambCell * \%cov * volSA}{equ} \tag{4.4}$$

Where $\%cov$ are the different values of sponge percentage cover (based on estimates of abundance). The amount of POC acquired per day (obtained from the carbon conversions) by the individual study species was included to calculate the average amount of POC consumed for the different values of sponge coverage in the study area per day according to the following equation:

$$CfilAr * \%cov * ArSA \tag{4.5}$$

Where $CfilAr$ is the POC filtered (mg C d^{-1}) per unit area of sponge, $\%cov$ is the sponge percentage cover (0.5 to 5%), and $ArSA$ is the total rocky reef area in the reserve (m^2). To obtain the *CfilAr*, the carbon acquired d^{-1} per sponge (with three specimens for each species) and the area (cm^2) of each sponge specimen, were divided to obtain the POC consumed normalised per unit area of sponge. Finally, since the ambient cell concentration (cells ml^{-1}) for the three picoplanktonic organisms detected is known, as is the amount of carbon present in each type of cell (from the carbon conversions), it was possible to calculate the amount of POC available in the study area as a result of the three groups of picoplankton. Using this value, the proportion of the total POC pool being consumed by the sponge assemblage in the reserve can be estimated with the following equation:

$$\frac{C\%cov * 100}{TC} \tag{4.6}$$

Where $C\%$ is the amount of prokaryotic POC consumed in the study area for the different values of sponge percentage cover (0.5 to 5%) and TC is the total amount of prokaryotic POC (g C d^{-1}) available in the study area estimated from the ambient concentrations of cells for all the study species.

4.2.9 Data analysis

4.2.9.1 Cell concentrations

For each sponge species, a Generalised Linear Model (GLM) was used to conduct an analysis of deviance with a quasibinomial error distribution (to correct for over- dispersion) and a log-link function to model inhalant cell concentration against exhalant cell concentration, and type of picoplankton (three levels: heterotrophic bacteria 'HetBact', *Prochlorococcus* 'Prochlo', *Synechococcus* 'Synecho'). Likelihood ratio tests were used to examine the hypothesis that a significant interaction occurred between the inhalant and exhalant current and picoplankton (in all cases $P < 0.05$). In the absence of significant interactions, the interaction term was removed and I concentrated on the main effects of inhalant-exhalant and picoplankton. To determine if there were significant differences in the ambient concentration of the three types of picoplankton present in the water column, a one-way analysis of variance (ANOVA) was performed. A post-hoc Tukey test was used to examine pairwise comparisons and data were pooled for the final calculations.

4.2.9.2 Retention efficiency

A one-way analysis of variance (ANOVA) was used to model retention efficiency (percentage retained between inhalant and exhalant water) against the type of picoplankton (three levels: HetBact, Prochlo, Synecho). The percentage data (retention) were arcsine-square root transformed to meet assumptions of normality and equal variance. The assumption of homogeneity of variance was examined using Bartlett's test ($P > 0.05$ in all cases). For the significant main effects ($P < 0.05$), a post-hoc Tukey test was used to examine pairwise comparisons and data were pooled for the final calculations. Statistical differences were determined at the 5% level and all statistical analyses were conducted by `R` ver. 2.10 (R Development 6 Core Team 2011).

4.3 Results

4.3.1 Cell concentrations and retention efficiency

Three populations of picoplanktonic organisms were identified that sponges removed from the ambient (inhalant) water: heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*-type cyanobacteria. Heterotrophic bacteria were the most abundant picoplanktonic cells, followed by *Prochlorococcus* and then *Synechococcus*. The average ambient cell concentration of heterotrophic bacteria was markedly higher ($4.4 \times 10^5 \pm 2.7 \times 10^5$ cells ml^{-1}) than that of *Prochlorococcus* ($7.2 \times 10^4 \pm 4.6 \times 10^4$ cells ml^{-1}) ($P < 0.001$) and *Synechococcus* ($1.9 \times 10^4 \pm 1.5 \times 10^4$ cells ml^{-1}) ($P < 0.001$). The range of cell concentrations measured in the water surrounding the different species are presented in Table 4.1. The GLM analysis of inhalant versus exhalant cell concentration and types of picoplankton yielded significant differences between the concentrations of cells found in the inhalant and exhalant currents for all of the study species, demonstrating the retention or removal of food particles by the sponge species. In general, the concentration of picoplanktonic organisms found in the ambient current of all the sponges remained similar throughout the sampling period (Fig. 4.1). The one-way ANOVA showed overall significant differences in the retention between *Prochlorococcus* and heterotrophic bacteria ($P < 0.001$), and *Synechococcus* and *Prochlorococcus* ($P = 0.05$). There was no significant difference in the retention between *Synechococcus* and heterotrophic bacteria (NS, $P = 0.1576$) by all the study species. The ranges of retention efficiency for the different picoplanktonic particles and sponge species are presented in Table 4.2. The large number of picoplanktonic cells that sponges can filter on a daily basis in a known volume of water are summarised in Table 4.3.

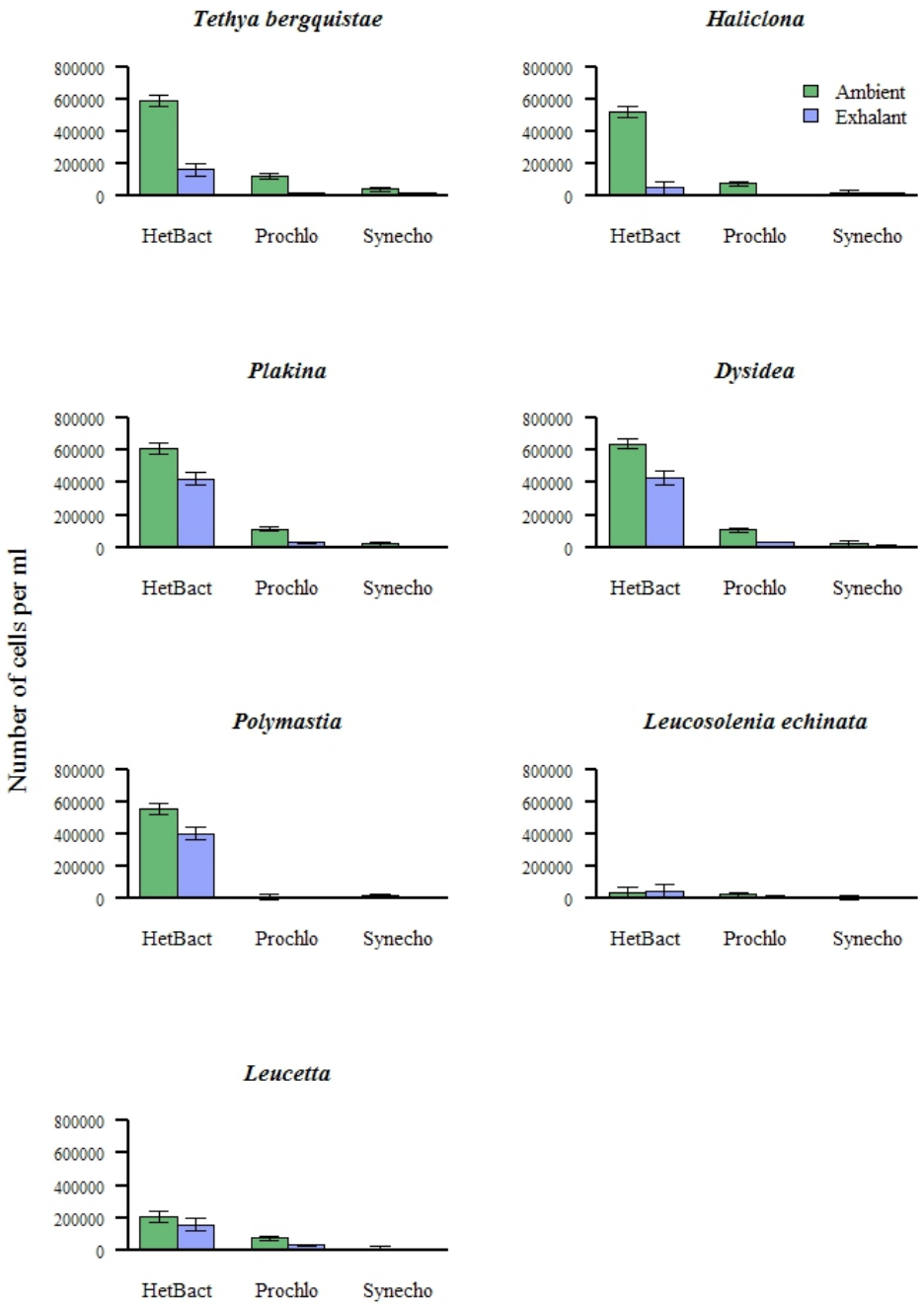


Figure 4.1: Plot showing inhalant versus exhalant cell concentrations and types of picoplankton (HetBact–heterotrophic bacteria, Prochlo–*Prochlorococcus*, Synecho–*Synechococcus*) obtained from the GLM analysis that yielded significant differences between the concentrations of cells found in the inhalant and exhalant current from all the study species.

Table 4.1: Ranges of ambient cell concentrations for the three types of picoplankton measured in the water surrounding the different study species. HetBact–Heterotrophic bacteria; Prochlo–*Prochlorococcus*; Synecho–*Synechococcus.*

Species	Number of cells in ambient water per ml^{-1}		
	HetBact	Prochlo	Synecho
Dysidea	7×10^5–8.3×10^5	1×10^5–1.2×10^5	2.1×10^4–2.8×10^4
Haliclona	4.6×10^5–5.7×10^5	7.5×10^4–8.4×10^4	2.1×10^4–2.3×10^4
Leucetta	6×10^4–1.6×10^5	4.7×10^4–5.3×10^4	5.9×10^3–7×10^3
Leucosolenia	5×10^4–6.6×10^4	1.8×10^4–2×10^4	2.5×10^3–2.7×10^3
Plakina	3.4×10^5–1×10^6	8.6×10^4–1.6×10^5	1.7×10^4–3.4×10^4
Polymastia	5×10^5–6.1×10^5	5.8×10^3–1.4×10^4	6.5×10^3–2.2×10^4
Tethya	5.3×10^5–6.3×10^5	9.9×10^4–1.5×10^5	2.4×10^4–6.8×10^4

4.3.2 Pumping rate of the study species

All sponge species removed the three types of picoplankton found in the ambient water with an overall removal efficiency of 40±14% for heterotrophic bacteria, 72 ± 11% for *Prochlorococcus*, and 54 ± 18% for *Synechococcus*. *T. bergquistae* had the highest flow rate of all the study species (111.4 ± 11.7 ml min^{-1}), followed by *Polymastia* sp., with an average flow rate of 110.4 ± 36.5 ml min^{-1}; the lowest flow rate measured was for *L. echinata* (32.6±8.0 ml min^{-1}). The retention efficiency, volume flow rate, and ambient concentration of each cell type were used to calculate the number of cells removed (ml min^{-1}) by each species (Table 4.3). All these values were used to estimate the amount of carbon acquired by the different sponge species from the picoplanktonic organisms they retained.

4.3.3 Picoplankton biomass retained and carbon acquired by sponges

Using habitat maps, the subtidal rocky reef area (considered the habitable substrate for sponges) within the study area was estimated to be 3.02 km^2, and the estimated volume of water in the area (calculated from bathymetry data) was 89,821 m^3 at high tide. Based on these figures and using the data from Equation 4.1, the total number of picoplanktonic cells present

Table 4.2: Ranges of retention efficiency for the three types of picoplankton removed by the seven study species. HetBact–Heterotrophic bacteria; Prochlo–*Prochlorococcus*; Synecho–*Synechococcus.*

Species	Retention efficiency		
	HetBact	Prochlo	Synecho
Dysidea	9–42%	54–96%	36–73%
Haliclona	88–91%	93–95%	18–43%
Leucetta	3–5%	56–59%	41–49%
Leucosolenia	0–66%	37–67%	66–75%
Plakina	9–41%	59–89%	51–81%
Polymastia	13–50%	52–91%	7–89%
Tethya	67–85%	83–94%	31–82%

in the water column of the study area at any point in time was calculated as; heterotrophic bacteria= $4 \times 10^{16} \pm 2.5 \times 10^{16}$ cells m^3, *Prochlorococcus* $= 6.4 \times 10^{15} \pm 4.1 \times 10^{15}$ cells m^3, and *Synechococcus* $= 1.7 \times 10^{15} \pm 1.3 \times 10^{15}$cells m^3. In a similar way, using Equation 4.2 the number of cells that the sponge assemblage would be capable of removing on a daily basis in the study area was estimated and the results are presented in Table 4.4. Using equation 4.3, I was able to calculate the average amount of POC consumed for the different values of sponge coverage in the study area per day (Fig. 4.2). The results from these calculations showed that in terms of carbon, heterotrophic bacteria were the primary carbon source for all sponges followed by *Prochloroccoccus* and then *Synechococcus*. Finally, the percentage of POC consumed by sponges from the total available prokaryotic POC in the form of each picoplanktonic organism in the study area was estimated using Equation 4.4. The results are presented in Table 4.5 for the different estimates of sponge abundance. Assuming a low sponge cover (1%), an assemblage would consume 0.2 % of the total available POC in the form of heterotrophic bacteria, 0.4% of *Prochlorococcus* and 0.3 % of *Synechococcus* per day. However, when assuming a high sponge cover (5%), an assemblage would consume 12.1% of the total POC available in the form of heterotrophic bacteria, 21.3 % of *Prochlorococcus* and 15.8 % of *Synechococcus* (Table 4.4) per day in the study area.

Table 4.3: Estimated mean flow rate, amount of water filtered and picoplanktonic cells removed by the study species over the sampling period. Data presented are averages (± StdDev), calculated for each sponge.

Species	Flow rate (ml min^{-1})	Number of cells removed (ml min^{-1})		
		HetBact	Prochlo	Synecho
Dysidea sp.	75.8 ± 50.5	$1.77 \times 10^7 \pm 1.97 \times 10^7$	$6.47 \times 10^6 \pm 6.02 \times 10^6$	$1.09 \times 10^6 \pm 9.45 \times 10^5$
Haliclona sp.	40.5 ± 20.6	$1.88 \times 10^7 \pm 9.02 \times 10^6$	$3.00 \times 10^6 \pm 1.55 \times 10^6$	$2.67 \times 10^5 \pm 1.62 \times 10^5$
Leucetta sp.	76.0 ± 30.6	$3.40 \times 105 \pm 1.57 \times 105$	$2.22 \times 10^6 \pm 9.27 \times 10^5$	$2.20 \times 10^5 \pm 4.28 \times 10^4$
Leucosolenia	32.6 ± 8.0	$7.31 \times 105 \pm 9.37 \times 105$	$3.37 \times 10^5 \pm 1.81 \times 10^5$	$6.00 \times 10^4 \pm 1.20 \times 10^4$
Plakina sp.	45.5 ± 22.9	$1.16 \times 10^7 \pm 1.67 \times 10^7$	$3.49 \times 10^6 \pm 1.54 \times 10^6$	$7.16 \times 10^5 \pm 4.07 \times 10^5$
Polymastia sp.	110.4 ± 36.5	$1.84 \times 10^7 \pm 1.89 \times 10^7$	$1.04 \times 10^6 \pm 7.36 \times 10^5$	$1.31 \times 10^6 \pm 1.13 \times 10^6$
Tethya	111.4 ± 11.7	$4.78 \times 10^7 \pm 6.57 \times 10^6$	$1.18 \times 10^7 \pm 1.80 \times 10^6$	$2.81 \times 10^6 \pm 2.85 \times 10^6$

Table 4.4: Summary of the number of cells filtered (cells d^{-1}) by sponge assemblages from each type of picoplankton retained. Values were calculated using a range of estimated abundances of sponge percentage cover in the study area.

Sponge cover (%)	Picoplankton (cells)		
	HetBact	Prochlo	Synecho
0.1	2.13×10^{18}	5.25×10^{17}	1.19×10^{17}
0.5	1.07×10^{19}	2.62×10^{18}	5.97×10^{17}
1.0	2.13×10^{19}	5.25×10^{18}	1.19×10^{18}
1.5	3.20×10^{19}	7.87×10^{18}	1.79×10^{18}
5.0	1.07×10^{20}	2.62×10^{19}	5.97×10^{18}

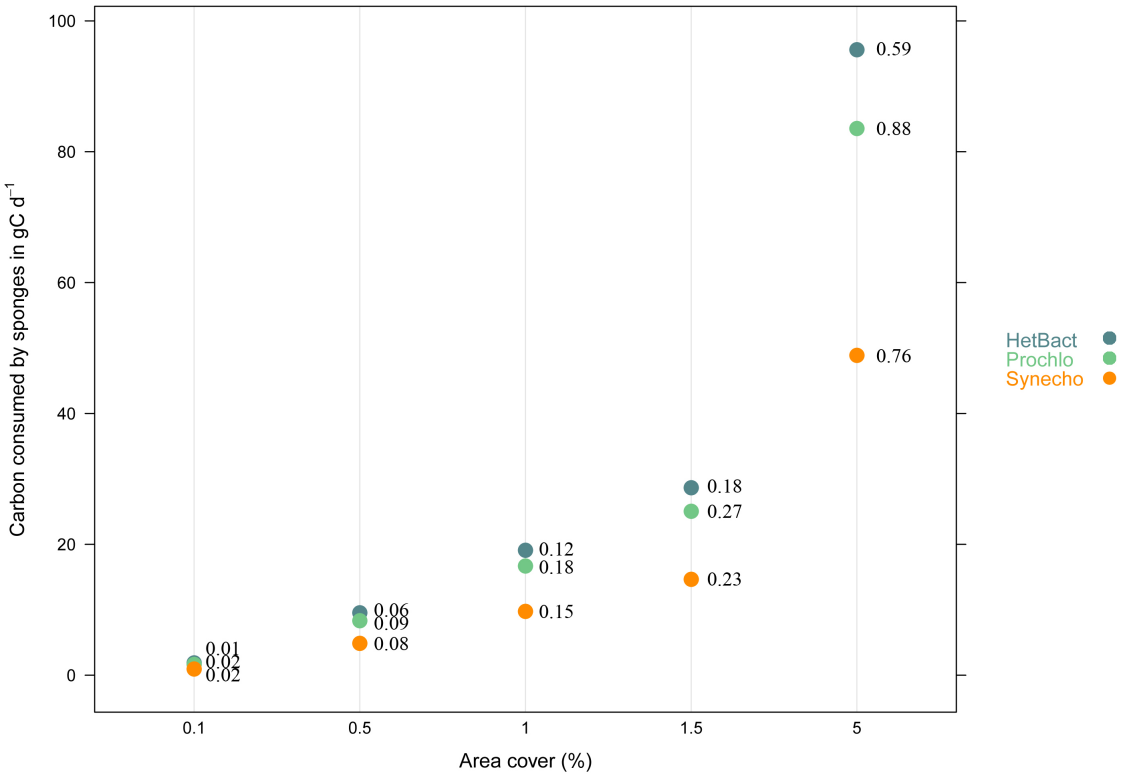


Figure 4.2: Carbon consumed by sponges (g C d^{-1}) in the area of the Taputeranga Marine Reserve from the three types of picoplanktonic organisms they retain. The graph shows a range of values for sponge percentage cover (0.1, 0.5, 1, 1.5 and 5%) measured at the site, as well as the percentage of POC consumed from the available POC within the MR. The values of the percentage of POC consumed by sponges from the total available in the MR are the numbers next to the blue, green and orange dots.

Table 4.5: Amount of POC consumed by sponges in the study area. Different levels of sponge cover for each picoplanktonic cell type are presented; as well as the percentage of the POC available as each of the three picoplanktonic cell types consumed by sponges. gCd^{-1} = grams of carbon per day; tgC = total grams of carbon

Sponge		HetBact			Prochlo			Synecho		
cover	gCd^{-1}	tgC	% POC consumed	gCd^{-1}	tgC	% POC consumed	gCd^{-1}	tgC	% POC consumed
0.1%	1.9	793.1	0.2	1.7	393.1	0.4	1.0	309.2	0.3
0.5%	9.6	793.1	1.2	8.4	393.1	2.1	4.9	309.2	1.6
1.0%	19.1	793.1	2.4	16.7	393.1	4.3	9.8	309.2	3.2
1.5%	28.7	793.1	3.6	25.1	393.1	6.4	14.7	309.2	4.7
5.0%	95.6	793.1	12.1	83.6	393.1	21.3	48.9	309.2	15.8

4.4 Discussion

In recent years, there has been an increasing interest in the role that benthic suspension-feeders play in the flow of energy between the water column and the benthos. However, most of these studies have focused on coral reefs (e.g. Richter et al. 2001; de Goeij & van Duyl 2007) and polar systems (e.g. Kowalke 1998; Gatti et al. 2002; Baird et al. 2004), with less attention given to temperate regions (but see Gardner & Thompson 2001; Helson et al. 2007; Pinkerton et al. 2008). Interestingly, fluxes in temperate and polar systems have been estimated to be higher than in tropical systems most likely because of higher productivity of the pelagic ecosystems in these regions (Pile et al. 1997; Pile & Young 2006). Since sponges are usually the dominant group (with the exception of corals on reefs) across different habitats worldwide, there have been a growing number of studies that have quantified the carbon flow as a result of sponge feeding activities. Previous studies based on *in situ* measurements of individual sponge species in temperate regions, have estimated carbon fluxes of 29 mg C m^2 d^{-1} in the sponge *Mycale lingua* (Pile et al. 1996), and 3.5 mg C m^2 d^{-1} in the sponge *Callyspongia* sp. (Hanson et al. 2009) from prokaryotic organisms. This study is the first to estimate the contribution of a sponge assemblage (rather than focusing on individual sponge species) to the carbon flow in a temperate rocky reef through the sponges' *in situ* feeding activities. Although it is recognised that there are more than just the seven species that were examined in the study area, the study species selected are by far the most abundant and represent $> 80\%$ of the available biomass. The results from this chapter show that the C consumption by sponges based on prokaryotes ranged from 70 to 3,500 mg C m^2 d^{-1} based on the minimum and maximum area estimates of 1% and 5% of sponge cover, respectively. To place these values in context, a study carried out in a shallow coastal region of the northern Wadden Sea by Baird et al. (2007), estimated that the total phytoplankton consumption by suspension-feeders was 387 mg C m^2 d^{-1} (based on a total area of 270 km^2). In the present study, a much smaller area (3.02 km^2) was considered and did not take into account other suspension-feeders (e.g. ascidians or bryozoans) that are present in the rocky reefs. Despite this, the values estimated in

the study are higher than previous studies. Therefore, I suggest that sponge assemblages have the potential to play a very important role in the transfer of POC from the water column to the benthos in the temperate marine ecosystem studied here, and potentially in other temperate sites.

4.4.1 Carbon flow through sponge assemblages in a defined study area

The results from this study indicate that sponge assemblages in the temperate rocky reef studied feed more efficiently on smaller cells (heterotrophic bacteria) than on bigger cells (*Prochlorococcus* and *Synechococcus*). Although heterotrophic bacteria were not retained as efficiently as the larger types of picoplankton (e.g. *Prochlorococcus*), their higher concentration in the water column meant that they contributed more to total C uptake without reflecting efficiency *per se*. Heterotrophic bacteria contributed the most to sponge diet with a C uptake of 95.6 g C d^{-1}, compared to the carbon uptake from *Prochlorococcus* (83.6 g C d^{-1}) and *Synechococcus* (48.9 g C d^{-1}). Thus, it seems that for the sponge assemblage studied here the relative importance of different picoplanktonic organisms as a food source is determined foremost by the concentration of these organisms in the surrounding water within which sponges are living (Huysecom et al. 1988), and secondarily by their ability to retain them. The findings from this study are in accordance with previous studies where heterotrophic bacteria have been found to be one of the primary sources of energy (in the form of particulate carbon) for sponges (Ribes et al. 1999b; Trussell et al. 2006; Yahel et al. 2007). In the present study a range of sponge abundance figures was used, since I know there are high levels of variability in sponge abundance across the study area, therefore it is difficult to accurately estimate overall sponge abundance across the study site. However, it is worth noting that in some areas of the reserve, sponge cover can reach $> 50\%$, so POC consumption could be much higher at a local scale, potentially even causing localised depletion.

The cell concentrations of the different picoplanktonic populations in the ambient water are similar to other studies that have investigated sponge feeding in temperate regions (Pile et al. 1996; Ribes et al. 1999b; Pile 2005; Jiménez & Ribes 2007). Previous studies have confirmed temporal

variation in pumping rates (Reiswig 1971b; Savarese et al. 1997), though this appears to be species-dependent (Bell et al. 1999). This is supported by the results presented here where different pumping rates were measured for different sponge species. It is important to note that the assumption of continuous pumping activity over a 24 hr period, is supported by recent findings by Pfannkuchen et al. (2009) who detected permanent pumping activity for several sponges *in situ*, using the method of tracer application for the detection of active pumping in sponges, which does not disturb the sponges and is free from experimental artefacts.

4.4.2 Sponges as trophic links in food webs

Carbon flows through food webs and can also be exchanged with the atmosphere (Azam 1998). The driving force of the carbon cycle is the primary production of organic matter by phytoplankton, which is essentially controlled by light intensity and the availability of nutrients (Wollast 1998). In marine food webs, bacteria are responsible for the recycling of nutrients to primary producers through the so-called 'microbial loop' (Azam 1983), and bacteria in the water column (picoplankton) can be utilised by various groups of suspension-feeders, including sponges. In this regard, there are numerous studies that have examined the importance of picoplankton as a food resource for different species of suspension-feeding invertebrates (see Table 4.6). The results from these studies show that these organisms can 'sidestep' the microbial loop by efficiently capturing picoplanktonic cells and directly utilising the organisms at the base of the microbial food web as a primary food source (Gili & Coma 1998). Examples of suspension-feeding organisms that have the ability to filter such particles are presented in Table 4.6, and include sponges, corals (Richter et al. 2001; Ribes et al. 2003), bivalves and ascidians (Ribes et al. 2005). The results from this study confirmed the assumption that sponge feeding represents a significant biomass link by coupling benthic and pelagic habitats. Furthermore, the results suggest that the fluxes of carbon provided from the micro-organisms they filter place sponges within an important functional group of organisms that link the pelagic microbial food web to the benthos (Reiswig 1974, 1975; Duckworth et al. 2006).

Table 4.6: Taxonomic groups of suspension-feeders and examples of studies where different species have been shown to feed on picoplanktonic cells (0.2–2 µm in size).

Group	Species	Source
Sponges	*Spongia officinalis*	Topçu et al. 2010
	Callyspongia sp.	Hanson et al. 2009
	Aphrocallistes vastus	Yahel et al. 2007
	Rhabdocalyptus dawsoni	
	Luffariella variabilis	Pile 2005
	Auletta constricta	
	Aplysilla sulphurea	
	Haliclona anonyma	Stuart & Klump 1984
Ascidians	*Phallusia julinea*	Pile 2005
	Halocynthia sp.	
	Didemnum sp.	
	Polycarpa pedunculata	
	Tridemnum solidum	Bak et al. 1998
	Ciona intestinalis	Petersen & Riisgård 1992
	Various species	Jørgensen et al. 1984
	Pyura stolonifera	Stuart & Klump 1984
Ciliates	*Strombidium sulcatum*	Christaki et al. 1998
	Uronema sp.	
Bivalves	*Mercenaria mercenaria*	Kach & Ward 2008
	Crassostrea virginica	
	Argopecten irradians	
Mussels	*Mytilus edulis*	Kach & Ward 2008
	Bathymodiolus childressi	Pile & Young 1999
	Aulacomya ater	Stuart & Klump 1984
	Choromytilus meridionalis	
	Perna perna	
Corals	*Corallium rubrum*	Picciano & Ferrier-Pages 2007
	Stylophora pistillata	Houlbeque et al. 2004
	Galaxea fascicularis	
	Tubastrea aurea	
	Madracis mirabilis	Bak et al. 1998
Polychaete	*Ditrupa arietina*	Sponges

In this study, the combined characteristics of the seven sponge species analysed were extrapolated to a defined study area, and the estimated volume of water (89,821 m^3), as well as the rocky reef area (3.02 km^2), allowed us to calculate the proportion of the available picoplanktonic C that sponge assemblages consume in this area. Because sponges are found worldwide and in high abundances in most hard substratum habitats, these organisms must be included in all energy flow models or food-web networks. This is important, since in some of these models, suspension-feeders provide an essential pathway for energy flow (see Whipple 1999; Baird et al. 2004). The construction of such food-web models gives quantitative information on the species and communities involved in marine systems, as well as their rates of consumption and production, dietary composition, and the flow of energy and materials between the system components (Baird et al. 2004). These models can then be incorporated in conservation, restoration, and management programmes. The data obtained from this study including biomass consumption (mg C m^{-2}), diet composition, carbon flow, and the feeding ecology of sponges, could be incorporated in these models in the future. This information will be important for future studies examining the ecological functioning of marine ecosystems, since understanding how changes in primary production or temperature impact ecosystems requires reliable models based on realistic representations of energy fluxes through ecosystems (Maury et al. 2007).

Since sponges play an important role in the balance and dynamics of carbon and nutrients in the water column (Díaz & Rutzler 2001), the results from this study represent an important step in developing a better understanding of the ecology of sponge-dominated assemblages on subtidal rocky reefs. Furthermore, this study shows that sponge assemblages are important components in temperate rocky habitats and that they play a key role in the transfer of energy from the water column to the benthos. This is particularly relevant since sponge feeding within the microbial loop could represent a significant biomass link with sponges being a sink for picoplankton (heterotrophic bacteria, *Prochlorococcus* and *Synechococcus*), and the linkages between sponges and the water column may have important implications for determining overall community structure (Menge et al. 1999). I also suggest that the effective use and substantial consumption of the picoplankton by

sponges might help to explain their ecological success and their capacity to reach high biomass in many marine systems.

In conclusion, this is the first study to estimate the contribution of a sponge assemblage (rather than focusing on individual sponge species) to the particulate carbon flow in a temperate rocky reef through the sponges feeding activity and it is the first such study for a temperate rocky subtidal community. In this study I demonstrated the clear importance of sponges in linking pelagic and benthic habitats. It remains to be elucidated if nutrient fluctuations and the abundance of picoplanktonic organisms present in the water column change over time (i.e. seasonally). These issues will be investigated and discussed in the following chapters (Chapter 5 and 6).

4.5 References

— Ayling A.L. (1981). The role of biological disturbance in temperate subtidal encrusting communities. Ecology, 62, 830-847.

— Azam F. (1998). Microbial control of oceanic carbon flux: The plot thickens. Science, 280, 694-696.

— Azam F., Fenchel T., Field J.G., Gray J.S., Meyerreil L.A. & Thingstad F. (1983). The ecological role of water-column microbes in the sea. Marine Ecology-Progress Series, 10, 257-263.

— Baird D., Asmus H. & Asmus R. (2004). Energy flow of a boreal intertidal ecosystem, the Sylt-Romo Bight. Marine Ecology-Progress Series, 279, 45-61.

— Baird D., Asmus H. & Asmus R. (2007). Trophic dynamics of eight intertidal communities of the Sylt-Romo Bight ecosystem, northern Wadden Sea. Marine Ecology-Progress Series, 351, 25-41.

— Becerro M.A., Turon X., Uriz M.J. & Templado J. (2003). Can a sponge feeder be a herbivore? *Tylodina perversa* (Gastropoda) feeding on *Aplysina aerophoba* (Demospongiae). Biological Journal of the Linnean Society, 78, 429-438.

— Bell A.H., Bergquist P.R. & Battershill C.N. (1999). Feeding biology of *Polymastia croceus*. Memoirs of the Queensland Museum, 44, 51-56.

— Bell J.J. (2008). The functional roles of marine sponges. Estuarine, Coastal and Shelf Science, 79, 341-353.

— Berman J. & Bell J. (2010). Spatial Variability of Sponge Assemblages on the Wellington South Coast, New Zealand. Open Marine Biology Journal, 4, 12-25.

— Bertilsson S., Berglund O., Karl D.M. & Chisholm S.W. (2003). Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea. Limnology and Oceanography, 48, 1721-1731.

— Cattaneo-Vietti R., Chiantore M., Misic C., Povero P. & Fabiano M. (1999). The role of pelagic-benthic coupling in structuring littoral benthic communities at Terra Nova Bay (Ross Sea) and in the Straits of Magellan. Scientia Marina, 63, 113-121.

— Charpy L. & Blanchot J. (1998). Photosynthetic picoplankton in French Polynesian atoll lagoons: estimation of taxa contribution to biomass and production by flow cytometry. Marine Ecology-Progress Series, 162, 57-70.

— Chisholm S.W., Frankel S.L., Goericke R., Olson R.J., Palenik B., Waterbury J.B., Westjohnsrud L. & Zettler E.R. (1992). *Prochlorococcus marinus* nov gen nov sp. - an oxyphototrophic marine prokaryote containing divinyl Chlorophyll a and chlorophyll b. Archives of Microbiology, 157, 297-300.

— Christaki U., Dolan J.R., Pelegri S. & Rassoulzadegan F. (1998). Consumption of picoplankton-size particles by marine ciliates: Effects of physiological state of the ciliate and particle quality. Limnology and Oceanography, 43, 458-464.

— Christaki U., Jacquet S., Dolan J.R., Vaulot D. & Rassoulzadegan F. (1999). Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnology and Oceanography, 44, 52-61.

— Coma R., Ribes M., Gili J.M. & Hughes R.N. (2001). The ultimate opportunists: consumers of seston. Marine Ecology-Progress Series, 219, 305-308.

— de Goeij J.M., Moodley L., Houtekamer M., Carballeira N.M. & van Duyl F.C. (2008). Tracing C-13-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM feeding. Limnology and Oceanography, 53, 1376-1386.

— de Goeij J.M. & van Duyl F.C. (2007). Coral cavities are sinks of dissolved organic carbon (DOC). Limnology and Oceanography, 52, 2608-2617.

— Díaz M.C. & Rutzler K. (2001). Sponges: An essential component of Caribbean coral reefs. Bulletin of Marine Science, 69, 535-546.

— Ducklow H.W., Kirchman D.L., Quinby H.L., Carlson C.A. & Dam H.G. (1993). Stocks and dynamics of bacterioplankton carbon during the spring bloom in the Eastern North Atlantic Ocean. Deep-Sea Research Part Ii-Topical Studies in Oceanography, 40, 245-263.

— Duckworth A.R., Bruck W.M., Janda K.E., Pitts T.P. & McCarthy P.J. (2006). Retention efficiencies of the coral reef sponges *Aplysina lacunosa*, *Callyspongia vaginalis* and *Niphates digitalis* determined by Coulter counter and plate culture analysis. Marine Biology Research, 2, 243-248.

— Gardner J.P.A. & Thompson R.J. (2001). Naturally low seston concentration and the net energy balance of the greenshell mussel (*Perna canaliculus*) at Island Bay, Cook Strait, New Zealand. New Zealand Journal of Marine and Freshwater Research, 35, 457-468.

— Gatti S., Brey T., Muller W.E.G., Heilmayer O. & Holst G. (2002). Oxygen microoptodes: a new tool for oxygen measurements in aquatic animal ecology. Marine Biology, 140, 1075-1085.

— Gili J.M. & Coma R. (1998). Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution, 13, 316-321.

— Gili J.M., Rossi S., Pages F., Orejas C., Teixido N., Lopez-Gonzalez P.J. & Arntz W.E. (2006). A new trophic link between the pelagic and benthic systems on the Antarctic shelf. Marine Ecology-Progress Series, 322, 43-49.

— Guillou L., Jacquet S., Chretiennot-Dinet M.J. & Vaulot D. (2001). Grazing impact of two small heterotrophic flagellates on Prochlorococcus and Synechococcus. Aquatic Microbial Ecology, 26, 201-207.

— Hanson C.E., McLaughlin M.J., Hyndes G.A. & Strzelecki J. (2009). Selective uptake of prokaryotic picoplankton by a marine sponge (*Callyspongia* sp.) within an oligotrophic coastal system. Estuarine Coastal and Shelf Science, 84, 289-297.

— Helson J.G., Pledger S. & Gardner J.P.A. (2007). Does differential particulate food supply explain the presence of mussels in Wellington Harbour (New Zealand) and their absence on neighbouring Cook Strait shores? Estuarine Coastal and Shelf Science, 72, 223-234.

— Herman P.M.J., Middelburg J.J., Van de Koppel J. & Heip C.H.R. (1999). Ecology of estuarine macrobenthos. Advances in Ecological Research, Vol 29, 29, 195-240.

— Houlbreque F., Tambutte E., Richard C. & Ferrier-Pages C. (2004). Importance of a micro-diet for scleractinian corals. Marine Ecology-Progress Series, 282, 151-160.

— Huysecom J., Richelle-Maurer E., Vyver G.V.D. & Vray B. (1988). Effect of bacterial concentration on retention and growth rate of the freshwater sponge *Ephydatia fluviatilis*. Physiological Zoology, 61, 535-542.

— Jiménez E. & Ribes M. (2007). Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges. Limnology and Oceanography, 52, 948-958.

— Jordana E., Charles F., Gremare A., Amouroux J.M. & Chretiennot-Dinet M.J. (2001). Food sources, ingestion and absorption in the suspension-feeding polychaete, *Ditrupa arietina* (O.F. Muller). Journal of Experimental Marine Biology and Ecology, 266, 219-236.

— Jørgensen C.B., Kiorboe T., Mohlenberg F. & Riisgard H.U. (1984). Ciliary and mucus-net filter feeding, with special reference to fluid mechanical characteristics. Marine Ecology-Progress Series, 15, 283-292.

— Kach D.J. & Ward J.E. (2008). The role of marine aggregates in the ingestion of picoplankton-size particles by suspension-feeding molluscs. Marine Biology, 153, 797-805.

— Kowalke J. (1998). Energy budgets of benthic suspension feeding animals of the Potter Cove (King George Island, Antarctica). Berichte zur Polarforschung, 0, I-VII, 1-147.

— Langdon C.J. & Newell R.I.E. (1990). Utilization of detritus and bacteria as food sources by 2 bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia-demissa*. Marine Ecology-Progress Series, 58, 299-310.

— Leon Y.M. & Bjorndal K.A. (2002). Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. Marine Ecology-Progress Series, 245, 249-258.

— Loh T.L. & Pawlik J.R. (2009). Bitten down to size: Fish predation determines growth form of the Caribbean coral reef sponge *Mycale laevis*. Journal of Experimental Marine Biology and Ecology, 374, 45-50.

— Lopez C.B., Cloern J.E., Schraga T.S., Little A.J., Lucas L.V., Thompson J.K. & Burau J.R. (2006). Ecological values of shallow-water habitats: Implications for the restoration of disturbed ecosystems. Ecosystems, 9, 422-440.

— Marie D., Brussaard C.P.D., Thyrhaug R., Bratbak G. & Vaulot D. (1999a). Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied Environmental Microbiology, 65, 45-52.

— Maury O., Faugeras B., Shin Y.J., Poggiale J.C., Ben Ari T. & Marsac F. (2007). Modeling environmental effects on the size-structured energy flow through marine ecosystems. Part 1: The model. Progress in Oceanography, 74, 479-499.

— Menge B.A. (2000). Top-down and bottom-up community regulation in marine rocky intertidal habitats. Journal of Experimental Marine Biology and Ecology, 250, 257-289.

— Menge B.A., Daley B.A., Lubchenco J., Sanford E., Dahlhoff E., Halpin P.M., Hudson G. & Burnaford J.L. (1999). Top-down and bottom-up regulation of New Zealand rocky intertidal communities. Ecological Monographs, 69, 297-330.

— Menge B.A., Daley B.A., Wheeler P.A., Dahlhoff E., Sanford E. & Strub P.T. (1997). Benthic-pelagic links and rocky intertidal communities: Bottom-up effects on top-down control? Proceedings of the National Academy of Sciences, 94, 14530-14535.

— Meylan A. (1988). Spongivory in Hawksbill turtles - a diet of glass. Science, 239, 393-395.

— Partensky F., Hess W.R. & Vaulot D. (1999). *Prochlorococcus*, a Marine Photosynthetic Prokaryote of Global Significance. Microbiology and Molecular Biology Reviews, 63, 106-127.

— Peters K.J., Amsler C.D., McClintock J.B., van Soest R.W.M. & Baker B.J. (2009). Palatability and chemical defenses of sponges from the western Antarctic Peninsula. Marine Ecology-Progress Series, 385, 77-85.

— Petersen J.K. & Riisgård H.U. (1992). Filtration capacity of the ascidian *Ciona intestinalis* and its grazing impact in a shallow fjord. Marine Ecology-Progress Series, 88, 9-17.

— Pfannkuchen M., Fritz G.B., Schlesinger S., Bayer K. & Brummer F. (2009). *In situ* pumping activity of the sponge *Aplysina aerophoba*, Nardo 1886. Journal of Experimental Marine Biology and Ecology, 369, 65-71.

— Picciano M. & Ferrier-Pages C. (2007). Ingestion of pico- and nanoplankton by the Mediterranean red coral *Corallium rubrum*. Marine Biology, 150, 773-782.

— Pile A.J. (1997). Finding Reiswig's missing carbon: quantification of sponge feeding using dual-beam flow cytometry. In: Proceedings of the 8th International Coral Reef Symposium, pp. 1403-1410.

— Pile A.J. (2005). Overlap in diet between co-occurring active suspension feeders on tropical and temperate reefs. Bulletin of Marine Science, 76, 743-749.

— Pile A.J., Patterson M.R., Savarese M., Chernykh V.I. & Fialkov V.A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnology and Oceanography, 42, 178-184.

— Pile A.J., Patterson M.R. & Witman J.D. (1996). *In situ* grazing on plankton <10 um by the boreal sponge *Mycale lingua*. Marine Ecology Progress Series, 141, 95-102.

— Pile A.J. & Young C.M. (2006). The natural diet of a hexactinellid sponge: Benthic-pelagic coupling in a deep-sea microbial food web. Deep-Sea Research Part I-Oceanographic Research Papers, 53, 1148-1156.

— Pinkerton M.H., Lundquist C.J., Duffy C.A.J. & Freeman D.J. (2008). Trophic modelling of a New Zealand rocky reef ecosystem using simultaneous adjustment of diet, biomass and energetic parameters. Journal of Experimental Marine Biology and Ecology, 367, 189-203.

— R Development Core Team, 2011. R: A Language and Environment for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

— Reiswig H.M. (1971b). Particle feeding in natural population of 3 marine demosponges. Biological Bulletin, 141, 568-591.

— Reiswig H.M. (1974). Water transport respiration and energetics of 3 tropical marine sponges. Journal of Experimental Marine Biology and Ecology, 14, 231-249.

— Reiswig H.M. (1975). Bacteria as food for temperate-water marine sponges. Canadian Journal of Zoology-Revue Canadienne De Zoologie, 53, 582-589.

— Ribes M., Coma R., Atkinson M.J. & Kinzie R.A. (2005). Sponges and ascidians control removal of particulate organic nitrogen from coral reef water. Limnology and Oceanography, 50, 1480-1489.

— Ribes M., Coma R. & Gili J.M. (1999a). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Ribes M., Coma R. & Gill J.M. (1999b). Seasonal variation of particulate organic carbon, dissolved organic carbon and the contribution of microbial communities to the live particulate organic carbon in a shallow near-bottom ecosystem at the Northwestern Mediterranean Sea. Journal of Plankton Research, 21, 1077-1100.

— Ribes M., Coma R. & Rossi S. (2003). Natural feeding of the temperate asymbiotic octocoral-gorgonian *Leptogorgia sarmentosa* (Cnidaria : Octocorallia). Marine Ecology-Progress Series, 254, 141-150.

— Richter C., Wunsch M., Rasheed M., Kotter I. & Badran M.I. (2001). Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature, 413, 726-730.

— Savarese M., Patterson M.R., Chernykh V.I. & Fialkov V.A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 1. *In situ* pumping rates. Limnology and Oceanography, 42, 171-178.

— Scanlan D. (2001). Cyanobacteria: ecology, niche adaptation and genomics. Microbiology Today, 28, 128-130.

— Stuart V. & Klumpp D.W. (1984). Evidence for food-resource partitioning by kelp-bed filter feeders. Marine Ecology Progress Series, 16, 27-37.

— Szyrmer J. & Ulanowicz R.E. (1987). Total flows in ecosystems. Ecological Modelling, 35, 123-136.

— Ting C.S., Rocap G., King J. & Chisholm S.W. (2002). Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. Trends in Microbiology, 10, 134-142.

— Trussell G.C., Lesser M.P., Patterson M.R. & Genovese S.J. (2006). Depth-specific differences in growth of the reef sponge *Callyspongia vaginalis*: role of bottom-up effects. Marine Ecology-Progress Series, 323, 149-158.

— Waterbury J.B., Watson S.W., Guillard R.R.L. & Brand L.E. (1979). Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature, 277, 293-294.

— Whipple S.J. (1999). Analysis of ecosystem structure and function: extended path and flow analysis of a steady-state oyster reef model. Ecological Modelling, 114, 251-274.

— Wollast R. (1998). Evaluation and comparison of the global carbon cycle in the coastal zone and in the open ocean. Global Coastal Ocean, 10, 213-252.

— Wright I.C., Mackay K., A. P., Gerring P., S. W., Goh A., Drury J. & Garlick R. (2006). Wellington South Coast - Habitat Mapping. In. unpublished map series Wellington.

— Wright J.T., Benkendorff K. & Davis A.R. (1997). Habitat associated differences in temperate sponge assemblages: The importance of chemical defence. Journal of Experimental Marine Biology and Ecology, 213, 199-213.

— Wulff J.L. (2006). Ecological interactions of marine sponges. Canadian Journal of Zoology, 84, 146-166.

— Yahel G., Marie D. & Genin A. (2005). InEx - a direct *in situ* method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnology and Oceanography, 3, 46-58.

— Yahel G., Sharp J.H., Marie D., Hase C. & Genin A. (2003). *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography, 48, 141-149.

— Yahel G., Whitney F., Reiswig H.M., Eerkes-Medrano D.I. & Leys S.P. (2007). *In situ* feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. Limnology and Oceanography, 52, 428-440.

Chapter 5

Utilisation of nutrients by sponge assemblages

Abstract

In this chapter, the nutrient fluxes of several sponge species were investigated in order to estimate the amount of nutrients that sponges remove from the water column (or release into it) at the assemblage level, as well as to increase our understanding of the availability and utilisation of nutrients over time. For the nutrient analysis (nitrate, nitrite, ammonium, phosphate and silicate), water samples were collected *in situ* from the ambient and exhalant current of several sponge species following the sampling methods used to measure the removal of food particles (see Chapter 2). A general pattern of uptake/release of a particular nutrient could not be detected across all sponges. The results yielded significant differences in nutrient concentrations between the inhalant-exhalant current of the water filtered by some of the species, but there was no consistent pattern. Only one of species, *Haliclona venustina*, exhibited ammonia excretion throughout the 2-yr period. In addition, there was no clear pattern associated with time of year and the nutrient levels measured in the water column. Nutrient concentrations in the water column varied spatially and temporally, suggesting high levels of temporal and spatial variation in nutrient availability. The significant assimilation and excretion of nitrate and nitrite by some of the study species may indicate microbial activity inside the sponges. If the assumption is that the sponge species studied here harbour associated symbionts, then it is possible that these temperate sponges could act as nitrifiers as sponges do in other tropical and temperate systems and that they contribute to nutrient recycling in the temperate subtidal rocky reef studied. As a result of sponge feeding and metabolism of particles, sponges excrete dissolved inorganic and organic waste back into the water column, and thus are major contributors to the cycling of essential elements.

5.1 Introduction

Nitrogen has been intensively studied due to its role as a limiting nutrient in the marine environment (e.g. Zehr & Ward 2002; Zehr & Kudela 2011); it influences the trophic biology and ecology of marine organisms and ultimately their ecological distribution and abundance (Fiore et al. 2010). Nitrogen can potentially be taken up as dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and particulate organic nitrogen (PON) (Davy et al. 2002). In seawater, there are three main forms of dissolved inorganic nitrogen (DIN): nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+). The level of dissolved inorganic nitrogen (DIN) in marine environments mainly depends on biological processes that constitute sinks and sources for these nutrients (Valiela 1984). Nitrate is the most important form of DIN in the oceans, and both ammonium and nitrate are generally considered to be the most important sources of regenerated and newly produced nitrogen for biological processes (Karl 2002; Suratman et al. 2008). Ammonium is often the most abundant form of inorganic nitrogen found in surface water layers following a period of productivity when the phytoplankton blooms have removed most of the nitrate and phosphate (Dugdale 1967). Soluble and particulate organic nitrogen compounds resulting from decaying organisms, together with nitrogen excreted by plants and animals, are rapidly broken down to ammonia (NH_3) by different types of bacteria, and ammonia is also excreted directly by animals together with urea and peptides (Grasshoff et al. 1999). Understanding ammonium consumption (e.g. by phytoplankton and nitrifying bacteria) and its generation by major food web components is very important, since estimates of the flux of ammonium through food webs from the biomass and metabolic activities of bacteria, phytoplankton, and zooplankton suggests that heterotrophic micro-organisms are responsible for most of the ammonium that is regenerated and assimilated by phytoplankton (Koike et al. 1986). Nitrification is the process of ammonium transformation to nitrite and subsequently to nitrate (Schlappy et al. 2010).

Nitrification mediated by sponges has been investigated in tropical (Díaz & Ward 997; Weisz et al. 2007) and temperate systems (Jiménez & Ribes 2007). In a coastal temperate system, sponges were found to nitrify at higher

rates than planktonic communities in productive waters, suggesting that sponges may be using alternative sources of nitrogen such as DON, and/or consuming associated bacteria (Jiménez & Ribes 2007). Sponges have also been found to assimilate and release DON and PON, as well as DIN in the form of ammonia, nitrite and nitrate (Corredor et al. 1988; Díaz & Ward 1997; Pile 1997; Bayer et al. 2008). On a coral reef in Australia, Davy et al. (2002) calculated the nitrogen flux between the sponge *Haliclona cymiformis* and its symbiotic rhodophyte partner by measuring ammonium uptake and excretion. They determined that the sponge-algal symbiosis can remove inorganic nitrogen from the surrounding water as ammonium and nitrate (most likely as a result of algal assimilation), and that the sponge releases waste ammonium that may potentially provide all of the nitrogen required by its rhodophyte partner. These studies present plausible data that nutrients are linked to all ecological processes and can interact with the benthos and the water column (Valiela 1984).

Phosphorus (P) is an essential nutrient utilised by all organisms for energy transport and growth (Benitez-Nelson 2000), and it enters the oceans predominantly through rivers (Benitez-Nelson 2000). In the ocean, total dissolved phosphorus is partitioned between a dissolved inorganic phosphorus pool (also referred to as soluble reactive phosphorus or phosphate, PO_4^{3-}), and a dissolved organic phosphorus pool (DOP) (Orrett et al. 1987). Dissolved nitrate and phosphate are important for ocean productivity; both nutrients become exhausted in the surface layers of the oceans through uptake by phytoplankton, and are vertically transported by upwelling or mixing processes (Dugdale 1967; Michaels et al. 1996; Tyrrell 1999). Silicate, a dissolved form of silicon (Si), is a major ocean nutrient and constitutes a fundamental nutrient for diatoms, silicoflagellates, radiolarians and many sponges (De la Rocha et al. 2000; Maldonado et al. 2005). These organisms are silicon biomineralisers that polymerise silica to build skeletons of biogenic silica (BSi) (De la Rocha et al. 2000; Maldonado et al. 2005). Recent studies have provided data suggesting that siliceous sponges play an important role in Si-cycling in diverse marine environments, with substantial contributions to the processes of BSi production and dissolution (Maldonado et al. 2005; Maldonado et al. 2010). While the evidence from these studies suggests that

the effects of silicate retention by sponges at the global-scale may be more important than previously thought, a numerical amount cannot be provided because of insufficient knowledge of global patterns of sponge BSi content and biomass distribution in the oceans (Maldonado et al. 2005).

The importance of suspension-feeders in marine ecosystems, and in particular the role they play through their filtering activities in controlling and maintaining water quality in aquatic systems is described in more detail in Chapter 4. Recent studies have demonstrated that sponges with large amounts of sponge-associated bacteria can utilise dissolved organic matter (DOM), but that in these cases this 'food source' supplies the majority of the carbon and energy needs of the sponge (Yahel et al. 2003; de Goeij et al. 2008). As also described in earlier chapters, sponges feed on suspended microorganisms such as heterotrophic and photosynthetic bacteria that constitute the 2–5 µm size fraction of the planktonic population. These picoplanktonic organisms are involved in the transformation and processing of dissolved inorganic nutrients before they become available to other marine organisms (Azam & Hodson 1977; Partensky et al. 1999). As a result of sponge feeding and metabolism of particles, sponges excrete dissolved inorganic and organic waste back into the water column, and thus are major contributors to the cycling of essential elements (Dame & Olenin 2005). These studies present plausible data that nutrients are linked to all ecological processes and can interact with the benthos and the water column (Valiela 1984).

Marine organisms utilise/need major nutrients such as phosphate, nitrate, ammonium and silicate for metabolic functions. In sponges, nutrients are acquired by phagocytosis of bacteria that are removed from the water column (Hentschel et al. 2002). The large biomass of sponges, their high filtration rates, and their associations with micro and macro-organisms, strongly suggest that they have an important role in the balance and dynamics of carbon and nutrients, and links between the water column and benthos (Díaz & Rutzler 2001). Due to the suspension-feeding activities of sponges, a certain number of transient bacteria are trapped within the vascular system, or stay attached to the sponge surface (Friedrich et al. 2001). Hence, in addition to a passing seawater microbial population serving as a food source (Hentschel et al. 2002); sponges harbour a great diversity of bacteria in their

tissues which can comprise as much as 40% of their biomass (Vacelet 1975; Vacelet & Donadey 1977). Various micro-organisms have evolved to reside in sponges, forming sponge–microbe associations. These micro-organisms include cyanobacteria, a diverse range of heterotrophic bacteria, facultative anaerobes and unicellular algae (Hentschel et al. 2002; Taylor et al. 2007; Mohamed et al. 2008). Sponge-associated prokaryotes are thought to mediate dissolved organic carbon (DOC) and to be capable of utilizing dissolved organic matter (DOM) from ambient water (Ribes et al. 1999a). Some studies have suggested that the symbiotic organisms can be a source of energy to sponges (Reiswig 1971b, 1975) and that they can contribute to the sponge' health and nutrition (Mohamed et al. 2010). Indeed, several lines of evidence indicate that some sponges obtain a significant portion of their nutrients from the bacterial symbionts (Wang 2006).

In this chapter, five nutrient fluxes were measured: nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_2). These nutrients were measured because they constitute important sinks and sources in the marine environment and because sponges are involved in the transformation and cycling process of dissolved inorganic nutrients before they become available to other marine organisms as mentioned above. Three main questions were addressed related to the utilisation of inorganic nutrients by 10 sponge species: (1) Do nutrient concentrations in the water column change over time? (2) Are there differences in the ambient nutrient concentration compared with the exhalant water for different sponge species? and (3) Does the flux identified in (2) show temporal variation for the different sponge species? The purpose of analysing water samples both in a single season multi-species survey and over time for three other species, was to provide *in situ* estimates of the utilisation of nutrients from a diversity of species (on a short-term basis including the species from the multi-species survey), and to investigate if there are differences in the use/production of nutrients over a 2-yr period.

5.2 Methods

5.2.1 Study site and species description

Nutrient fluxes of sponge assemblages were examined at two sites located within the Taputeranga Marine Reserve on the south coast of Wellington, New Zealand. The study area was described in previous chapters emphasising the high abundance and diversity of sponges that are found living in the rocky subtidal ecosystems that characterise this coast. For the present study, seven sponge species were used to examine the amount of nutrients removed by sponges from the water column at the assemblage level (i.e. a multi-species survey). These species were *Dysidea* sp., *Haliclona* sp., *Plakina* sp., *Polymastia* sp., *Tethya bergquistae*, *Leucetta* sp., and *Leucosolenia echinata*. In addition, three common and conspicuous demosponges were selected for a seasonal study (*Haliclona venustina*, *Strongylacidon* sp., and *Crella incrustans*). All the species were chosen because they are very common in the study area and their well defined exhalant oscula reduced the risk of sampling error and made sampling *in situ* easier.

The seasonal study species *Haliclona venustina* is often found on vertical rock walls on the sides of channels, and boulders; it is yellow coloured, has big well defined oscula ($\sim 4-5$ mm diameter), and it can be seen with finger-like outgrowths rising from the basal mass. *Crella incrustans* is a very conspicuous encrusting species found on boulders, rock walls, and crevices; it has a bright orange-red colour and a smooth surface with raised and well defined oscula ($\sim 4-5$ mm diameter). *Strogylacidon* sp. is commonly found on boulders and rock walls; it has well defined oscula ($\sim 3-4$ mm diameter) and a smooth surface, with a blue-grey colour (Berman et al. 2008) (see species photos in Appendix A).

5.2.2 Sampling

Two separate sets of seawater samples were collected: samples for a multi-species survey and samples collected at three different times per year (winter, spring, and autumn) over a 2-yr period (2009 and 2010). Both surveys were collected across the two sites. It was not possible to collect samples during

winter time in the second year due to bad weather conditions, and a few methodological problems were encountered with some samples during collection and during the nutrient analysis; hence no summer data were available. The multi-species survey was carried out during the high tide of the sampling days in: November 2008, January, February and March 2009 and included the seven sponge species listed above. All samples were collected *in situ* using SCUBA from specimens found at 7 to 10 meters depth. Ambient and exhalant water samples were collected from three specimens of all the study species following the same sampling method described for the flow cytometry samples (see Chapter 2); the only difference here was that 10-ml sterile plastic syringes with blunt-ended needles were used. Sample collection consisted of water being slowly drawn from the inhalant current at a distance of ~ 3 cm from the sponge ostia, and then from the exhalant water inside the oscular aperture; care was taken to avoid contact with sponge tissue. Immediately after collection, water samples were transferred to 15-ml sterile polypropylene test tubes, kept in the dark, transported on ice and frozen (-20 °C) in an upright position until analysis.

5.2.3 Nutrient analysis

On the day of analysis, samples were thawed at room temperature, with the exception of those for silicate analysis that were kept in the dark at 4 °C for 24 hr prior to analysis. This was because dissolved silicate is reported to polymerise or crystallise during the freezing process; samples have to be given sufficient time for depolymerisation to occur (Kirkwood 1994). All glassware used for the analyses was washed with a phosphate-free detergent and acid rinsed (10% diluted HCl). The precision of each analysis depends on measuring the exact weight of chemical/reagent to make up the working solutions, so considerable care was taken during this process. The working stock solution, as well as the working standards, were prepared fresh daily. The determination of ammonium is very sensitive to traces of this nutrient in the laboratory so all the samples were covered with parafilm during the analysis. All samples were measured for inorganic nutrient content with a SAN^{plus} Segmented Flow Analyser following SKALAR methods (Skalar,

Breda, The Netherlands). Unfortunately, the seasonal samples collected for silicate analysis could not be measured due to serious problems with the silicate analysis resulting in an unresolved technical failure of the machine. An increase or decrease in the exhalant concentration of nutrients (compared to the ambient concentration) was interpreted as the uptake or excretion/release or uptake/use, respectively of that particular nutrient by a sponge.

5.2.4 Data analysis

For each of the seven sponge species from the multi-species survey, a paired t-test was used to evaluate differences between inhalant and exhalant water nutrient levels (data met assumptions of normality and equal variance). This was done separately for the five different nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, SiO_2), where nutrient concentrations were expressed in µM. The Shapiro-Wilk test was used to examine the assumption of normality. To analyse the samples collected over the 2-yr period, a paired t-test was conducted where each sponge species was analysed separately for each nutrient per season. Statistical differences were determined at the 5% level and all statistical analyses were conducted by `R` ver. 2.10 (R Development 6 Core Team 2011).

5.3 Results

5.3.1 Changes in nutrient fluxes in the water column over time

The levels of dissolved inorganic nutrients in the ambient water that was collected from around the sponges varied greatly between samples and over time during the 2-yr study period. Nitrate concentrations ranged from 0.39 to 1.81 µM (spring and winter of the first year, respectively). Nitrite generally showed very low levels over the study period (range from 0.05 to 0.46 µM); the highest levels of nitrite measured from the ambient water were from spring of the first year (0.64 ± 0.48 µM). Ammonium levels ranged from 2.08 to 9.50 µM (spring of the first year and autumn of the second year, respectively). Phosphate concentrations remained low during the study period, ranging from 0.25 to 0.34 µM, except in autumn of the second year when the levels of this nutrient were very high (9.02 µM). Looking at the results in more detail,

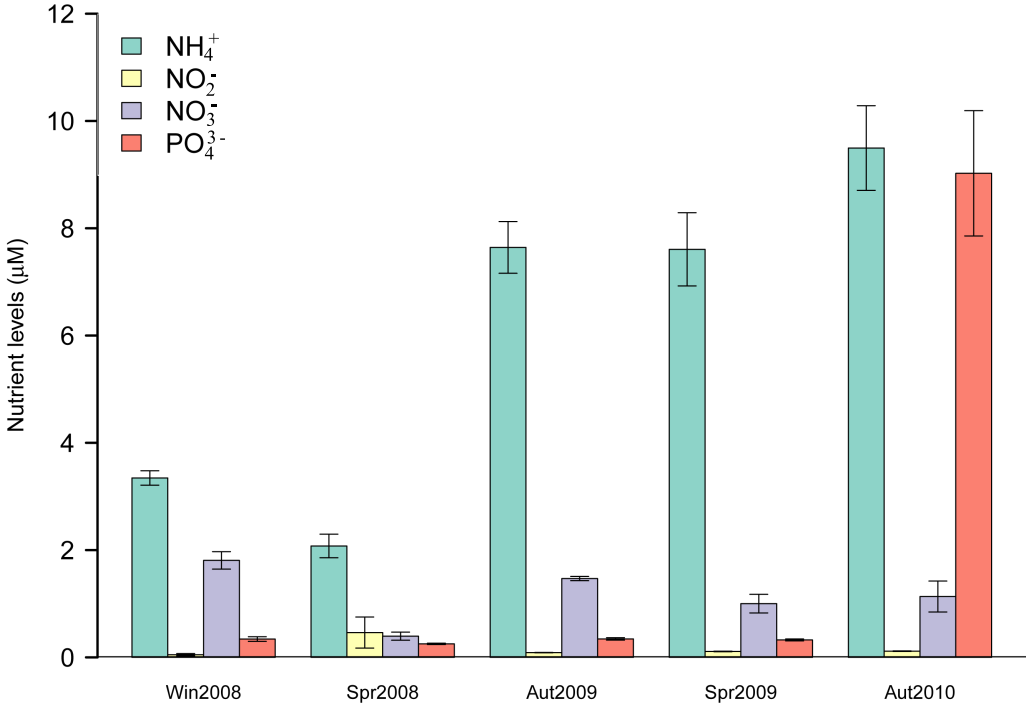


Figure 5.1: Seasonal variation in the ambient nutrient levels (µM) over the 2-yr study period.

the highest concentration of nitrate measured from the ambient water was in winter of the first year (2.07 ± 0.23 µM), and the highest levels of ammonium and phosphate from the ambient water were detected during autumn of the second year (10.56 ± 1.34; 10.47 ± 0.79 µM, respectively). The average nutrient concentrations across the 2-year study period in the ambient seawater were: 1.16 µM of nitrate, 0.17 µM of nitrite, 6.03 µM of ammonium and 2.06 µM of phosphate (Fig. 1).

5.3.2 Nutrient fluxes for the multi-species survey

The results from the multi-species survey yielded significant differences in nutrient levels between the ambient and exhalant water for some, though not all, of the species (Table 1). These differences were detected in the ammonium levels of *Leucetta* sp., which showed an increase of this nutrient in its exhalant water, along with *Plakina* sp. and *Haliclona* sp. In addition, the nitrate levels from the ambient water around *T. bergquistae* decreased.

The phosphate levels were higher in the exhalant water from *Haliclona* sp., and *Plakina* sp. (Table 1). There were no further significant differences.

Table 5.1: Fluxes of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_2) from the inhalant and exhalant current of the sponge species. Nutrient levels are expressed in µM and data presented are averages ($n = 3$, ± StdDev). Results from the one-way analysis of variance are also shown for the interaction between sponge species and nutrients. Significance at the 5% level

Species	*Inh*	*Exh*	Statistic / ANOVA
		NO_3^-	
T. bergquistae	1.27 ± 0.05	0.48 ± 0.02	$F_{1,4} = 960.05$; $P <$ **0.001**
Haliclona sp.	0.59 ± 0.05	0.55 ± 0.07	$F_{1,4} = 0.95$; $P = 0.38$
Plakina sp.	0.64 ± 0.10	0.66 ± 0.04	$F_{1,4} = 0.11$; $P = 0.76$
Dysidea sp.	0.65 ± 0.08	0.69 ± 0.05	$F_{1,4} = 0.37$; $P = 0.57$
Polymastia sp.	1.30 ± 0.05	1.32 ± 0.11	$F_{1,4} = 0.04$; $P = 0.84$
L. echinata	0.41 ± 0.05	0.39 ± 0.01	$F_{1,4} = 0.17$; $P = 0.69$
Leucetta sp.	0.42 ± 0.01	0.43 ± 0.03	$F_{1,4} = 0.14$; $P = 0.72$
		NO_2^-	
T. bergquistae	0.09 ± 0.00	0.13 ± 0.02	$F_{1,4} = 12.32$; $P <$ **0.01**
Haliclona sp.	0.07 ± 0.01	0.08 ± 0.01	$F_{1,4} = 1.86$; $P = 0.24$
Plakina sp.	0.03 ± 0.01	0.02 ± 0.02	$F_{1,4} = 0.03$; $P = 0.87$
Dysidea sp.	0.02 ± 0.00	0.03 ± 0.02	$F_{1,4} = 0.08$; $P = 0.78$
Polymastia sp.	0.07 ± 0.01	0.12 ± 0.06	$F_{1,4} = 2.44$; $P = 0.19$
L. echinata	0.01 ± 0.00	0.01 ± 0.00	$F_{1,4} = 2.92$; $P = 0.16$
Leucetta sp.	0.01 ± 0.00	0.02 ± 0.01	$F_{1,4} = 0.37$; $P = 0.57$
		NH_4^+	
T. bergquistae	7.33 ± 1.76	9.77 ± 1.72	$F_{1,4} = 2.97$; $P = 0.16$
Haliclona sp.	6.78 ± 0.72	15.99 ± 2.77	$F_{1,4} = 31.17$; $P <$ **0.001**
Plakina sp.	5.95 ± 0.34	8.76 ± 0.84	$F_{1,4} = 28.51$; $P <$ **0.001**
Dysidea sp.	6.05 ± 0.41	8.44 ± 2.01	$F_{1,4} = 4.09$; $P = 0.11$
Polymastia sp.	5.73 ± 0.93	6.01 ± 1.25	$F_{1,4} = 0.09$; $P = 0.77$

continued on next page...

Species	*Inh*	*Exh*	Statistic / ANOVA
L. echinata	6.96 ± 0.42	6.8 ± 0.68	$F_{1,4} = 0.11$; $P = 0.75$
Leucetta sp.	6.06 ± 0.78	8.15 ± 0.88	$F_{1,4} = 9.56$; $P <$ **0.01**
		PO_4^{3-}	
T. bergquistae	0.44 ± 0.01	0.45 ± 0.02	$F_{1,4} = 0.34$; $P = 0.59$
Haliclona sp.	0.05 ± 0.02	1.86 ± 0.58	$F_{1,4} = 29.35$;$P <$ **0.001**
Plakina sp.	0.37 ± 0.05	0.39 ± 0.07	$F_{1,4} = 0.21$; $P = 0.67$
Dysidea sp.	0.37 ± 0.00	0.4 ± 0.05	$F_{1,4} = 1.49$; $P = 0.29$
Polymastia sp.	0.82 ± 0.10	0.78 ± 0.04	$F_{1,4} = 0.27$; $P = 0.63$
L. echinata	0.56 ± 0.03	0.58 ± 0.01	$F_{1,4} = 0.87$; $P = 0.40$
Leucetta sp.	0.58 ± 0.01	0.57 ± 0.01	$F_{1,4} = 4.58$; $P = 0.09$
		SiO_2	
T. bergquistae	0.96 ± 0.02	0.92 ± 0.05	$F_{1,4} = 1.38$; $P = 0.30$
Haliclona sp.	1.36 ± 0.01	0.32 ± 0.11	$F_{1,4} = 39.8$; $P <$ **0.001**
Plakina sp.	1.32 ± 0.02	1.18 ± 0.01	$F_{1,4} = 142$; $P <$ **0.001**
Dysidea sp.	0.96 ± 0.03	0.96 ± 0.01	$F_{1,4} =$ 2e-04; $P = 0.99$
Polymastia sp.	0.93 ± 0.01	0.92 ± 0.01	$F_{1,4} = 2.98$; $P = 0.16$
L. echinata	0.70 ± 0.01	0.65 ± 0.03	$F_{1,4} = 8.00$; $P <$ **0.01**
Leucetta sp.	0.64 ± 0.00	0.61 ± 0.02	$F_{1,4} = 9.46$; $P <$ **0.01**

5.3.3 Changes in nutrient fluxes over time

The utilisation of nutrients by sponges varied with time of year. For *C. incrustans*, the paired t-test indicated significantly higher nitrite levels in exhalant waters in winter, but lower exhalant levels in spring and autumn of the second year. Also, a significantly higher level of ammonium was detected in the exhalant water compared to ambient water in the spring of the first year. Finally, the analysis for this species showed significantly higher phosphate levels in the exhalant water in spring of the first year and significantly lower exhalant levels in spring of the second year (Fig. 2, Table 2). For *H. venustina*, the paired t-test indicated significantly higher exhalant levels of

nitrate in autumn of the first year and significantly lower exhalant nitrite levels in spring and autumn of the second year. For this species significantly higher exhalant ammonium levels were observed in spring and autumn of the first year. The analysis also showed significantly higher exhalant phosphate levels in spring and autumn of the first year (Fig. 3, Table 3). For *Strongylacidon* sp., the paired t-test indicated significantly higher exhalant levels of nitrate in spring and autumn of the first year. This species also exhibited significantly lower exhalant nitrite levels in spring of the second year and during autumn of both years. Significantly lower exhalant levels of ammonium were detected in winter and significantly higher exhalant levels of ammonium were detected in spring and autumn of the first year. Finally, the analysis for *Strongylacidon* sp. showed significantly lower phosphate levels during winter and significantly higher levels in spring of the second year and autumn of the first year (Fig. 5, Table 4). No other significant differences were observed between ambient and exhalant nutrient levels.

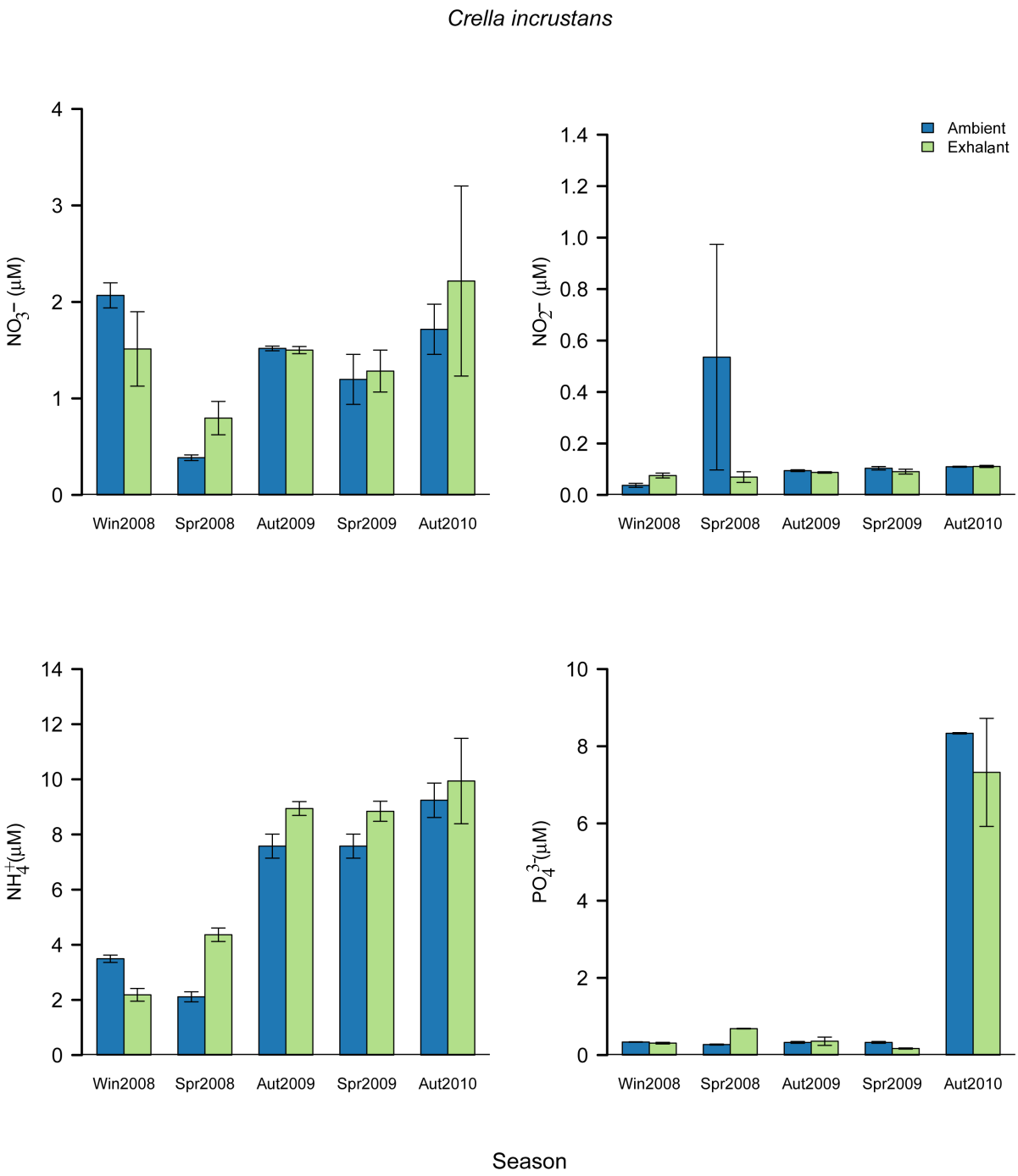


Figure 5.2: Dissolved nutrient concentrations (µM) from ambient (inhalant) and exhalant water samples taken from *Crella incrustans* showing increase or decrease ($n = 3$, ± StdDev) of each nutrient over time.

Table 5.2: Paired t test showing differences between inhalant and exhalant water nutrient levels (µM) for each season and each of the inorganic nutrients studied for *Crella incrustans*. Data presented are averages (± StdDev). Significance at the 5% level, df = 2

Crella incrustans					
Nutrient	Season	Inh	Exh	t	P
NO_3^-	Win2008	2.07 ± 0.23	1.51 ± 0.67	1.11	0.38
	Spr2008	0.39 ± 0.05	0.80 ± 0.30	−2.42	0.13
	Spr2009	1.20 ± 0.45	1.28 ± 0.38	−1.94	0.19
	Aut2009	1.52 ± 0.04	1.50 ± 0.06	1.32	0.31
	Aut2010	1.72 ± 0.45	2.22 ± 1.71	−0.68	0.56
NO_2^-	Win2008	0.04 ± 0.01	0.08 ± 0.02	−7.00	< 0.05
	Spr2008	0.54 ± 0.76	0.07 ± 0.04	1.03	0.40
	Spr2009	0.10 ± 0.01	0.09 ± 0.02	4.58	< 0.05
	Aut2009	0.09 ± 0.01	0.09 ± 0.00	5.23	< 0.05
	Aut2010	0.11 ± 0.00	0.11 ± 0.01	−0.19	0.86
NH_4^+	Win2008	3.49 ± 0.23	2.18 ± 0.40	3.58	0.06
	Spr2008	2.11 ± 0.32	4.36 ± 0.42	−11.94	< 0.01
	Spr2009	7.58 ± 0.76	8.84 ± 0.63	−2.59	0.12
	Aut2009	7.58 ± 0.76	8.94 ± 0.43	−3.81	0.06
	Aut2010	9.24 ± 1.08	9.94 ± 2.69	−0.52	0.64
PO_4^{3-}	Win2008	0.34 ± 0.01	0.31 ± 0.04	1.06	0.39
	Spr2008	0.27 ±0.02	0.69 ± 0.01	−22.03	< 0.01
	Spr2009	0.33 ± 0.04	0.17 ± 0.03	4.62	< 0.05
	Aut2009	0.33 ± 0.04	0.36 ± 0.19	−0.30	0.78
	Aut2010	8.34 ± 0.03	7.32 ± 2.43	0.71	0.54

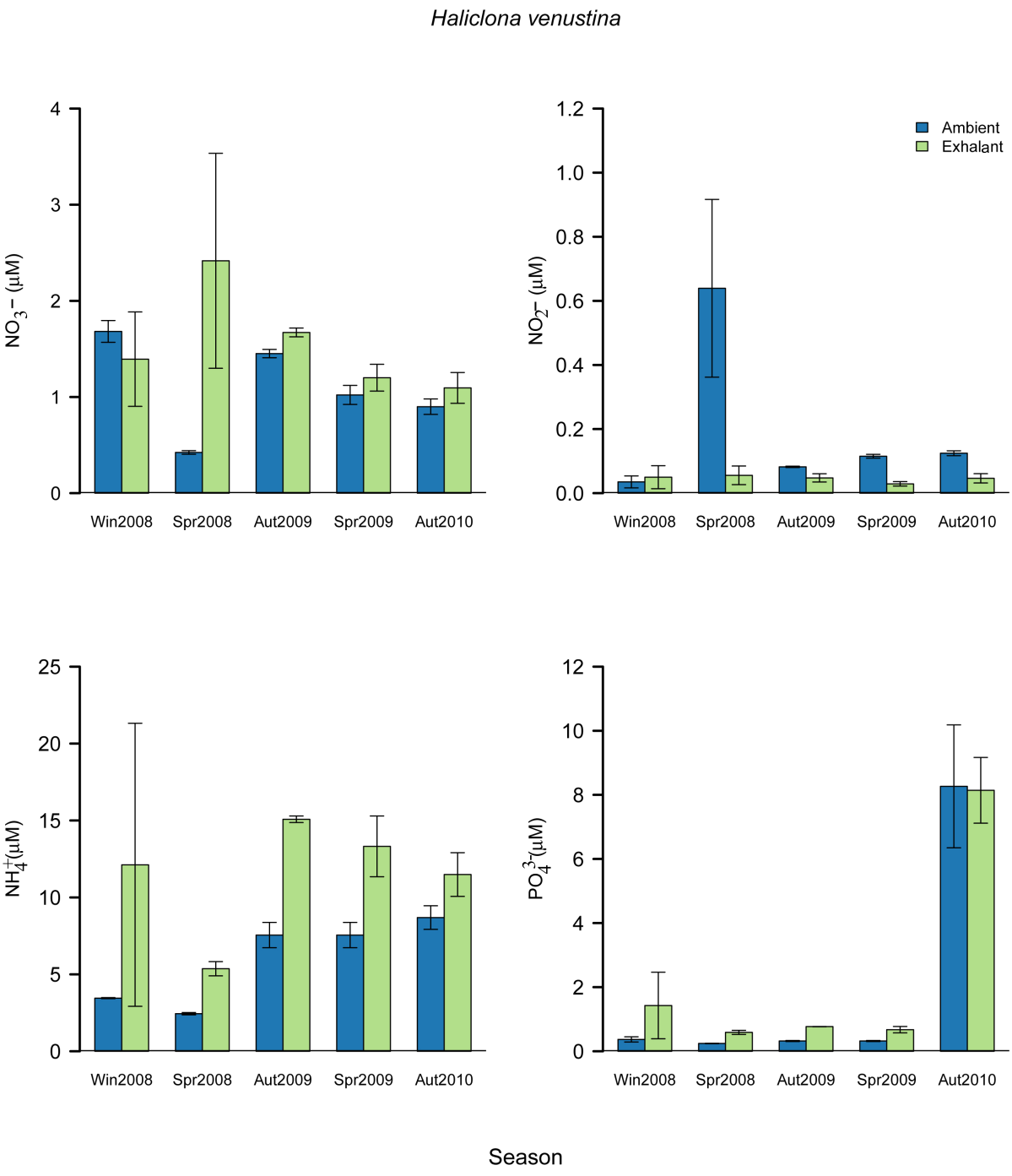


Figure 5.3: Dissolved nutrient concentrations (µM) from ambient (inhalant) and exhalant water samples taken from *Haliclona venustina* showing increase or decrease (n= 3, ± StdDev) of each nutrient over time.

Table 5.3: Paired t test showing differences between inhalant and exhalant water nutrient levels (µM) for each season and each of the inorganic nutrients studied for *Haliclona venustina*. Data presented are averages (± StdDev). Significance at the 5% level, df = 2

Haliclona venustina					
Nutrient	Season	Inh	Exh	t	P
NO_3^-	Win2008	1.68 ± 0.20	1.39 ± 0.85	0.51	0.65
	Spr2008	0.42 ± 0.03	2.42 ± 1.94	−1.75	0.22
	Spr2009	1.02 ± 0.17	1.20 ± 0.24	−2.01	0.18
	Aut2009	1.45 ± 0.07	1.67 ± 0.08	−4.92	< 0.05
	Aut2010	0.90 ± 0.14	1.09 ± 0.28	−2.43	0.13
NO_2^-	Win2008	0.04 ± 0.03	0.05 ± 0.06	−0.84	0.48
	Spr2008	0.64 ± 0.48	0.06 ± 0.05	2.20	0.15
	Spr2009	0.12 ± 0.01	0.03 ± 0.01	88.46	< 0.001
	Aut2009	0.08 ± 0.00	0.05 ± 0.02	2.32	0.14
	Aut2010	0.12 ± 0.01	0.05 ± 0.03	10.35	< 0.01
NH_4^+	Win2008	3.45 ± 0.06	12.12 ± 15.93	−0.93	0.44
	Spr2008	2.44 ± 0.13	5.37 ± 0.80	−6.74	< 0.05
	Spr2009	7.55 ± 1.42	13.32 ± 3.42	−2.65	0.11
	Aut2009	7.55 ± 1.42	15.08 ± 0.37	−7.46	< 0.05
	Aut2010	8.69 ± 1.33	11.49 ± 2.46	−3.17	0.08
PO_4^{3-}	Win2008	0.37 ± 0.14	1.43 ± 1.80	−1.00	0.42
	Spr2008	0.24 ± 0.01	0.59 ± 0.11	−5.48	< 0.05
	Spr2009	0.32 ± 0.03	0.67 ± 0.17	−3.78	0.06
	Aut2009	0.32 ± 0.03	0.77 ± 0.01	−20.59	< 0.01
	Aut2010	8.27 ± 3.32	8.14 ± 1.78	0.04	0.969

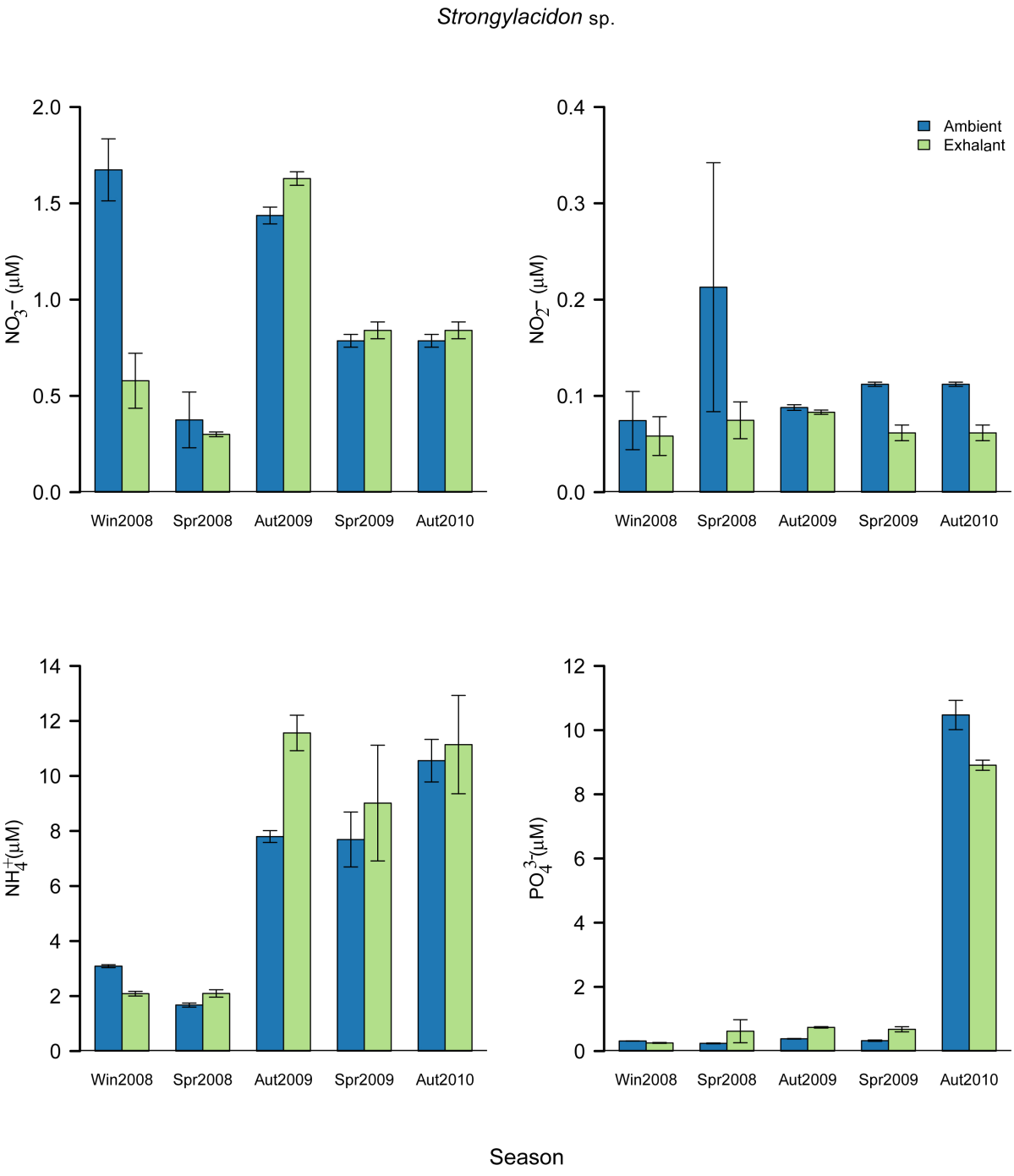


Figure 5.4: Dissolved nutrient concentrations (µM) from ambient (inhalant) and exhalant water samples taken from *Strongylacidon* sp. showing increase or decrease (n= 3, ± StdDev) of each nutrient over time.

Table 5.4: Paired t test showing differences between inhalant and exhalant water nutrient levels (µM) for each season and each of the inorganic nutrients studied for *Strongylacidon* sp. Data presented are averages (± StdDev). Significance at the 5% level, df = 2

Strongylacidon sp.					
Nutrient	Season	Inh	Exh	t	P
NO_3^-	Win2008	1.67 ± 0.28	0.58 ± 0.25	52.26	< 0.001
	Spr2008	0.38 ± 0.25	0.30 ± 0.02	0.47	0.67
	Spr2009	0.79 ± 0.06	0.84 ± 0.08	−1.73	0.22
	Aut2009	1.44 ± 0.08	1.63 ± 0.06	−6.88	< 0.05
	Aut2010	0.79 ± 0.06	0.84 ± 0.08	−1.14	0.36
NO_2^-	Win2008	0.07 ± 0.05	0.06 ± 0.03	0.32	0.77
	Spr2008	0.21 ± 0.22	0.07 ± 0.03	0.94	0.44
	Spr2009	0.11 ± 0.00	0.06 ± 0.01	7.91	< 0.05
	Aut2009	0.09 ± 0.01	0.08 ± 0.00	6.30	< 0.05
	Aut2010	0.11 ± 0.00	0.06 ± 0.01	6.03	< 0.05
NH_4^+	Win2008	3.09 ± 0.09	2.09 ± 0.15	10.43	< 0.01
	Spr2008	1.67 ± 0.13	2.09 ± 0.24	−4.25	0.05
	Spr2009	7.69 ± 1.73	9.01 ± 3.64	−0.45	0.69
	Aut2009	7.80 ± 0.37	11.56 ± 1.11	−5.65	< 0.05
	Aut2010	10.56 ± 1.34	11.14 ± 3.10	−0.24	0.83
PO_4^{3-}	Win2008	0.31 ± 0.01	0.25 ± 0.02	5.14	< 0.05
	Spr2008	0.24 ± 0.02	0.62 ± 0.62	−1.02	0.41
	Spr2009	0.33 ± 0.04	0.68 ± 0.14	−4.38	< 0.05
	Aut2009	0.38 ± 0.02	0.74 ± 0.04	−19.40	< 0.01
	Aut2010	10.47 ± 0.79	8.91 ± 0.27	3.61	0.06

5.4 Discussion

In this study, sponges were treated as a 'black-box' where the nutrient fluxes for individual sponges were studied, rather than the total amounts released/taken-up or the mechanisms by which this happens. These will be a focus of future studies. The uptake or release of nutrients from/to the water that sponges filter was measured *in situ*. Significant differences in the uptake and/or release of some of the dissolved nutrients for most of the species in the multi-species survey were found, as well as differences in the uptake and release of nutrients were also found for the three sponge species studied seasonally across the two–year period. However, no general patterns of uptake/release of a particular nutrient could be detected across all sponge species or time intervals.

Sponges ingest nitrogen with their food and, like many other marine invertebrates they usually excrete ammonium as a metabolic waste product (Brusca & Brusca 1990). Considering the large amount of food particles (picoplankton) that sponges remove from the water column (see results section in Chapter 4), a large efflux or release of ammonium was expected. However, only *H. venustina, Plakina* sp. and *Leucetta* sp. from the multi-species survey had significantly higher exhalant ammonium levels compared to ambient water. From the seasonal study, *C. incrustans* showed a significant release of this nutrient during spring of the first year, *H. venustina* showed a significant release of ammonium during spring and autumn of the first year, while *Strongylacidon* sp. only excreted ammonium during spring and autumn of the first year. Photosynthetically active micro-organisms, such as cyanobacteria and eukaryotic algae, in addition to other micro-organisms, are often located in the outer-light-exposed tissue layers of sponges (Rüztler 1985; Wilkinson 1992); it is possible that the activity of these organisms may be important in explaining the fluxes reported in this study. The lack of ammonium excretion by some sponges, or at certain times of year, could be indicative of different microbial populations in different sponge species, and/or changes in the activities of these microbial consortia in response to changes in the environment (e.g. seasonal temperature). In those cases where no ammonium release was detected, it may be that these associated microbes

are taking up all the waste ammonium and either assimilating it or converting it to nitrite/nitrate. However, the results did not show evidence of release of nitrite/nitrate for species that did not produce ammonium. This is perhaps surprising and it is not currently possible to explain this result without further consideration of the specific microbial communities associated with these sponges.

T. bergquistae and *Strongylacidon* sp. were the species that showed lower nitrate levels in the exhalant water compared to the inhalant water. Again this is likely to be explained by the activity of microbes assimilating this nutrient. *C. incrustans* was the only species that exhibited release of nitrite. Given that bacteria and archaea are the only organisms able to oxidize ammonium (Francis et al. 2005; You et al. 2009); the significant excretion of nitrite by *C. incrustans* suggests that this species may harbour micro-organisms that are responsible for the nitrite flux observed (Jiménez & Ribes 2007). However, it remains to be elucidated if the three species mentioned above are photosynthetic sponges or not, although there are studies that have determined the presence of phototrophic micro-organisms in other sponges (See Gaino et al. 2006; Lemloh et al. 2009; Sipkema & Blanch, 2010). In contrast, *H. venustina* released nitrate during autumn of the first year, while *Strongylacidon* sp. released nitrate in autumn of the first year indicating the presence of nitrifying bacteria. This release of nitrate and nitrite can be interpreted as evidence that these sponges may be active sites of nitrification (Corredor et al. 1988; Díaz & Ward 1997). Nitrite levels were lower in the exhalant water of *C. incrustans*, *H. venustina* and *Strongylacidon* sp. at different times of the year (spring and autumn 2009 and autumn 2010), while *Strongylacidon* sp. released nitrate during autumn of the first year. In this case, it is possible that nitrite might be removed and used by nitrifying bacteria to produce nitrate. While it is possible to explain the lack of differences in N levels between inhalant and exhalant water by sponges stopping pumping, which in turn may have reduced the oxygen concentrations in the sponge creating an anaerobic environment suitable for denitrification (see Hoffmann et al. 2009; Schlappy et al 2010), this is unlikely. Measurements of exhalant flow/pumping rates were conducted for most of these species (see Chapter 4) with no evidence of regular pumping inactivation.

It was mentioned earlier (see introduction 5.1), that sponges are hosts to nitrogen-fixing symbionts. Symbiotic nitrifying bacteria metabolise ammonium excreted by the sponges and other symbionts by oxidizing it to nitrate (Corredor et al. 1988). In a recent study, Mohamed et al. (2010) demonstrated that nitrification in sponges is a metabolic capacity of the sponges' symbionts. In the present study the symbiotic associations were not examined; nevertheless, the significant uptake and release of DIN compounds, and especially nitrate and nitrite, by some of the study species may indicate microbial activity inside the sponges.

The analyses detected silicate uptake only for *Plakina* sp. and *Haliclona* sp., which is perhaps surprising. It is, however, important to mention that our samples were not filtered (to remove diatoms from seawater) prior to storage, therefore it is possible that our silicate analyses failed to detect silicate uptake by other species that are heavily skeletonised sponges such as *Tethya* sp. and *Polymastia* sp., because the amount they consumed was very low as to compensate the silicate increase in the stored water samples resulting from diatom dissolution. Very little is known about silicate uptake rates and retention by sponges, although a recent study showed that the retention of a dense bathyal population of hexatinellid sponges was substantial, and that silicate silicate retention of a dense temperate sponge was far from negligible (Maldonado et al. 2005). Other authors have suggested that silica uptake is intimately linked to processes governing sponge growth (Reincke & Barthel 1997), while it is also affected by factors such as seasonality (Turon et al. 1998). One of the aims of the current study was to examine the natural fluctuations in silica in the water column over time. However, due to complications with the auto-analyser (see methods) this analysis could not be performed. Nevertheless, the results obtained from the multi-species survey suggest that some of the species are using silicate, which is likely to be for spicule synthesis. As expected the calcareous sponges did not show evidence of silica uptake. More interannual studies of BSi production in the field are needed in order to provide a more realistic quantification of the use of silicate by sponges (Maldonado et al. 2005).

There were no clear trends in the nutrient concentrations in the water column (ambient water) over time, suggesting high levels of temporal and

spatial variation. There was, however, one pattern observed in the levels of phosphate measured: the levels of this nutrient in the water column were significantly higher in autumn of the second year than at other times. Since phosphate enters the ocean mainly through rivers, this spike of phosphate levels in autumn could be a pulse in the environment; it is possible that during that time of the year, there was lots of rainfall and the massive spike was due to storm water outfalls. Also levels of ammonia in the water column were significantly lower during the spring and winter of the first year and significantly higher during autumn of the second year, while the levels of nitrate in the water column were significantly lower during spring of the first year than at other times. It is possible that a lack of clear pattern of nutrient uptake/release of nutrients in some of the study species, and the fact that not all species showed significant uptake/release at different times of the year, may be related to high levels of temporal fluctuations in parameters such as water temperature, sponge size, concentration of food and oxygen in the water column (Ayling 1983; Cloern 1996; Ribes et al. 1999b).

To conclude, the nitrate and nitrite excretion results reported here for some sponge species, suggest organic nitrogen mineralization by the sponge's metabolism and the presence of micro-organisms inside the sponges that may play a role in nitrification (Díaz & Ward 1997). However, future research in the study area is needed to examine the putative symbiotic associations using microscopy and molecular techniques in order to verify their presence. Other studies have suggested that temperate sponges are important nitrifiers in other regions. If the sponge species studied here do harbour microbial symbionts, it is likely that they might be nitrifiers as in other temperate systems and therefore, contribute to nutrient recycling in temperate subtidal rocky reefs of New Zealand. However, more work on the physiology and ecology of any microbial partners is needed in order to fully understand the nutrient fluxes observed here and their ecological importance.

5.5 References

— Ayling A.L. (1983). Factors affecting the spatial distributions of thinly encrusting sponges from temperate waters. Oecologia, 60, 412-418.

— Azam F. & Hodson R.E. (1977). Size distribution and activity of marine microheterotrophs. Limnology and Oceanography, 22, 492-501.

— Bayer K., Schmitt S. & Hentschel U. (2008). Physiology, phylogeny and *in situ* evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*. Environmental Microbiology, 10, 2942-2955.

— Benitez-Nelson C.R. (2000). The biogeochemical cycling of phosphorus in marine systems. Earth-Science Reviews, 51, 109-135.

— Berman J., Perea-Blázquez A., Kelly M. & Bell J. (2008). Sponges of the Wellington south coast. In: The Taputeranga Marine Reserve (eds. Gardner J & Bell J). First Edition Ltd Wellington, pp. 225-236.

— Brusca R.C. & Brusca G.J. (1990). Phylum Porifera: the sponges. Sinauer Press, Sunderland, MA, USA.

— Cloern J.E. (1996). Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. Reviews of Geophysics, 34, 127-168.

— Corredor J.E., Wilkinson C.R., Vicente V.P., Morell J.M. & Otero E. (1988). Nitrate release by Caribbean reef sponges. Limnology and Oceanography, 33, 114-120.

— Dame R.F. & Olenin S. (2005). Comparative Roles of Suspension-Feeders in Ecosystems. Kluwer Academic Publishers, Dordrecht, The Netherlands.

— Davy S.K., Trautman D.A., Borowitzka M.A. & Hinde R. (2002). Ammonium excretion by a symbiotic sponge supplies the nitrogen requirements of its rhodophyte partner. Journal of Experimental Marine Biology, 205, 3505-3511.

— de Goeij J.M., Moodley L., Houtekamer M., Carballeira N.M. & van Duyl F.C. (2008). Tracing C-13-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM feeding. Limnology and Oceanography, 53, 1376-1386.

— De la Rocha C.L., Brzezinski M.A. & DeNiro M.J. (2000). A first look at the distribution of the stable isotopes of silicon in natural waters. Geochimica Et Cosmochimica Acta, 64, 2467-2477.

— Díaz M.C. & Rutzler K. (2001). Sponges: An essential component of Caribbean coral reefs. Bulletin of Marine Science, 69, 535-546.

— Díaz M.C. & Ward B.B. (1997). Sponge-mediated nitrification in tropical benthic communities. Marine Ecology-Progress Series, 156, 97-107.

— Dugdale R.C. (1967). Nutrient limitation in the sea: dynamics, identification, and significance. Institute of Marine Science, University of Alaska, 33, 685-695.

— Fiore C.L., Jarett J.K., Olson N.D. & Lesser M.P. (2010). Nitrogen fixation and nitrogen transformations in marine symbioses. Trends in Microbiology, 18, 455-63.

— Francis C.A., Roberts K.J., Beman J.M., Santoro A.E. & Oakley B.B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America, 102, 14683-14688.

— Friedrich A.B., Fischer I., Proksch P., Hacker J. & Hentschel U. (2001). Temporal variation of the microbial community associated with the mediterranean sponge *Aplysina aerophoba*. FEMS Microbiology Ecology, 38, 105-113.

— Gaino E., Sciscioli M., Lepore E., Rebora M. & Corriero G. (2006). Association of the sponge *Tethya orphei* (Porifera, Demospongiae) with filamentous cyanobacteria. Invertebrate Biology, 125, 281-287.

— Grasshoff K., Ehrhardt M., Kremling K. & Anderson L.G. (1999). Methods of Seawater Analysis. Wiley-VCH.

— Hentschel U., Hopke J., Horn M., Friedrich A.B., Wagner M., Hacker J. & Moore B.S. (2002). Molecular evidence for a uniform microbial community in sponges from different oceans. Applied and Environmental Microbiology, 68, 4431-4440.

— Hoffmann L. (1999). Marine cyanobacteria in tropical regions: diversity and ecology. European Journal of Phycology, 34, 371-379.

— Jiménez E. & Ribes M. (2007). Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges. Limnology and Oceanography, 52, 948-958.

— Karl D.M. (2002). Nutrient dynamics in the deep blue sea. Trends in Microbiology, 10, 410-418.

— Kirkwood D.S. (1994). The SANplus segmented flow analyzer. Seawater analysis. In. Skalar Analytical B.V. De Breda, The Netherlands, p. 51.

— Koike I., Holm-Hansen O. & Biggs D.C. (1986). Inorganic nitrogen metabolism by Antarctic phytoplankton with special reference to ammonium cycling. Marine Ecology, 30, 105-116.

— Lemloh M.-L., Fromont J., Brummer F. & Usher K.M. (2009). Diversity and abundance of photosynthetic sponges in temperate Western Australia. BMC Ecol, 9, 4.

— Maldonado M., Carmona C., Velasquez Z., Puig A., Cruzado A., Lopez A. & Young C.M. (2005). Siliceous sponges as a silicon sink: An overlooked aspect of benthopelagic coupling in the marine silicon cycle. Limnology and Oceanography, 50, 799-809.

— Maldonado M., Riesgo A., Bucci A. & Rüztler K. (2010). Revisiting silicon budgets at a tropical continental shelf: Silica standing stocks in sponges surpass those in diatoms. Limnology and Oceanography, 55, 2001-2010.

— Michaels A.F., Olson D., Sarmiento J.L., Ammerman J.W., Fanning K., Jahnke R., Knap A.H., Lipschultz F. & Prospero J.M. (1996). Inputs, losses and transformations of nitrogen and phosphorus in the pelagic North Atlantic Ocean. Biogeochemistry, 35, 181-226.

— Mohamed N.M., Colman A.S., Tal Y. & Hill R.T. (2008). Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. Environmental Microbiology, 10, 2910-2921.

— Mohamed N.M., Saito K., Tal Y. & Hill R.T. (2010). Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. Isme Journal, 4, 38-48.

— Orrett K. & Karl D.M. (1987). Dissolved organic phosphorus production in surface seawaters. Limnology and Oceanography, 32, 383-395.

— Partensky F., Blanchot J. & Vaulot D. (1999). Differential distribution and ecology of Prochlorococcus and Synechococcus in oceanic waters: A review. Bulletin de l'Institut Oceanographique (Monaco), 0, 457-475.

— Pile A.J. (1997). Finding Reiswig's missing carbon: quantification of sponge feeding using dual-beam flow cytometry. In: Proceedings of the 8th International Coral Reef Symposium, pp. 1403-1410.

— Reincke T. & Barthel D. (1997). Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. Marine Biology, 129, 591-593.

— R Development Core Team, 2011. R: A Language and Environment for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

— Reiswig H.M. (1971b). Particle feeding in natural population of 3 marine demosponges. Biological Bulletin, 141, 568-591.

— Reiswig H.M. (1975). Bacteria as food for temperate-water marine sponges. Canadian Journal of Zoology-Revue Canadienne De Zoologie, 53, 582-589.

— Ribes M., Coma R. & Gili J.M. (1999a). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Ribes M., Coma R. & Gill J.M. (1999b). Seasonal variation of particulate organic carbon, dissolved organic carbon and the contribution of microbial communities to the live particulate organic carbon in a shallow near-bottom ecosystem at the Northwestern Mediterranean Sea. Journal of Plankton Research, 21, 1077-1100.

— Rüztler K. (1985). Associations between Caribbean sponges and photosynthetic organisms. In: New Perspectives in Sponge Biology (ed. Rüztler K). Smithsonian Institution Press Washington, D.C., USA, pp. 455-466.

— Schlappy M.L., Schottner S.I., Lavik G., Kuypers M.M.M., de Beer D. & Hoffmann F. (2010). Evidence of nitrification and denitrification in high and low microbial abundance sponges. Marine Biology, 157, 593-602.

— Sipkema D. & Blanch H.W. (2010). Spatial distribution of bacteria associated with the marine sponge *Tethya californiana*. Marine Biology, 157, 627-638.

— Suratman S., Jickells T., Weston K. & Fernand L. (2008). Seasonal variability of inorganic and organic nitrogen in the North Sea. Hydrobiologia, 610, 83-98.

— Taylor M.W., Radax R., Steger D. & Wagner M. (2007). Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiology and Molecular Biology Reviews, 71, 295-347.

— Turon X., Tarjuelo I. & Uriz M.J. (1998). Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defence. Functional Ecology, 12, 631-639.

— Tyrrell T. (1999). The relative influences of nitrogen and phosphorus on oceanic primary production. Nature, 400, 525-531.

— Vacelet J. (1975). Electron-microscope study of association between bacteria and sponges of genus Verongia (Dictyoceratida). Journal De Microscopie Et De Biologie Cellulaire, 23, 271-&.

— Vacelet J. & Donadey C. (1977). Electron-microscope study of association between some sponges and bacteria. Journal of Experimental Marine Biology and Ecology, 30, 301-314.

— Valiela I. (1984). Marine ecological processes. Springer-Verlag, New York, N.Y., USA.

— Wang G.Y. (2006). Diversity and biotechnological potential of the sponge-associated microbial consortia. Journal of Industrial Microbiology & Biotechnology, 33, 545-551.

— Weisz J.B., Hentschel U., Lindquist N. & Martens C.S. (2007). Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. Marine Biology, 152, 475-483.

— Wilkinson C.R. (1992). Symbiotic interactions between marine sponges and algae. In: Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored. (ed. Reisser W). Biopress Ltd. Bristol, England, pp. 113-151.

— Yahel G., Sharp J.H., Marie D., Hase C. & Genin A. (2003). *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography, 48, 141-149.

— You J., Das A., Dolan E.M. & Hu Z.Q. (2009). Ammonia-oxidizing archaea involved in nitrogen removal. Water Research, 43, 1801-1809.

— Zehr J.P. & Kudela R.M. (2011). Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. Annual Review of Marine Science, Vol 3, 3, 197-225.

— Zehr J.P. & Ward B.B. (2002). Nitrogen cycling in the ocean: New perspectives on processes and paradigms. Applied and Environmental Microbiology, 68, 1015-1024.

Chapter 6

Temporal fluctuations in food utilisation by three common species of demosponges

Abstract

Previous studies have found that cold and temperate ecosystems exhibit a marked seasonal variation in environmental conditions that strongly affects the dynamics of benthic organisms. Spatial and temporal changes in food quality, quantity and availability affect important processes, such as growth, metabolism and the spatial distribution of benthic-suspension feeding organisms. In this chapter, the temporal variation of the food particles (picoplanktonic organisms) present in the water column and the consumption rates by three demosponges (*Crella incrustans*, *Haliclona venustina* and *Strongylacidon* sp.) were examined. Three sampling times over a two-year period were used to examine temporal changes in particle retention efficiency, relative to their abundance in the water column. The sampling of water carried out over time allowed a realistic measurement of changes in the concentration of picoplanktonic organisms over time. The major findings from this chapter were that the picoplanktonic species composition in the water column changed seasonally and inter-annually, and that there were temporal differences in the proportions of the different types of picoplankton consumed by the study species. Sponges consumed different proportions of picoplanktonic cells in relation to their concentration in the water column, and therefore the amount of carbon gained by the sponges changed temporally. Although the retention efficiencies of heterotrophic bacteria by the three sponge species were similar to those of *Prochlorococcus* and *Synechococcus*, heterotrophic bacteria were the most abundant food source throughout the 2-yr study period and represented the main source of carbon for the study species. This suggests that sponges do select food particles

that optimise their nutritional intake. A marked seasonal variation in food resources and consumption of carbon was not observed for the study species. However, the fact that these sponges are obtaining their major carbon source from the same type of picoplankton all year round implies that in terms of carbon use, the sponges get sufficient energy from consuming heterotrophic bacteria to meet metabolic demands. These estimates support the conclusion that sponges actively select food particles that optimise their nutritional intake, but it remains to be elucidated if the picoplankton identified in this study constitutes major food sources or if there are other particles that supply more carbon destined for the biological processes of the study species. Also, since the food particle abundance identified in this study remains the same all year round, further research is needed in order to determine if the study species exhibit seasonal differences in growth.

6.1 Introduction

In the marine environment, changes in the population dynamics of phytoplankton have significant implications for ecosystem functioning and are the main biological trigger of seasonal variation (Bavestrello et al. 2006). The population dynamics of the phytoplankton can be interpreted as a response to changes in individual processes that regulate the biomass (in measures such as carbon, nitrogen, or chlorophyll concentration), species composition, and the spatial distribution of the phytoplankton (Cloern 1996). Nearly half of global primary production occurs in the oceans where the phytoplankton shows considerable seasonal variation (Cloern & Jassby 2008). An example of such seasonal patterns are phytoplankton blooms which are events of rapid production and accumulation of phytoplankton biomass in response to increased sunlight, water column stability, changing physical forces such as tides, winds or rainfall and river runoff (Cloern 1996). Phytoplankton blooms in spring are a well known phenomenon in temperate waters (Cadee 1986). These blooms or changes in phytoplankton biomass, affect the biochemical composition and reactivity of the suspended particulate matter and synthesis of organic matter required for the reproduction and growth of heterotrophs including bacteria, zooplankton, and benthic organisms (Cloern 1996).

Phytoplankton biomass responds quickly to environmental fluctuations because algal cells have the capacity to divide daily under optimal conditions (Cloern & Jassby 2008). In polar and temperate latitudes the abundance of

phytoplankton shows cyclic variation (Boero et al. 1996), and it is possible to recognise the involvement of the phytoplankton dynamics at every level of trophic chains from shallow to deep water (Bavestrello et al. 2006). In shallow waters, productivity variation elicits a seasonal pattern in the activity of benthic organisms that implies a very rapid response to fluctuations in the food supply to the benthos (Graf 1989). In temperate areas, benthic organisms in shallow waters have more immediate access to planktonic production because of their proximity to the photic layer, and because of tidal or wind vertical mixing (Gili et al. 1998). The feeding activity of benthic suspension-feeders is highly influenced by the species composition and the intensity of phytoplankton blooms (Beukema & Cadee 1991). Localised low levels of primary production result in food limitation to suspension-feeders (Helson et al. 2007) and since these benthic organisms graze on phytoplankton; changes in productivity can affect the production and transport of particulate food to the benthos (Graf et al. 1982).

Cold and temperate ecosystems exhibit a marked seasonal variation in environmental conditions that strongly affects the dynamics of benthic organisms (Coma & Ribes 2003). Similarly, spatial and temporal changes in food quality, quantity and availability affect important processes such as the growth, metabolism and spatial distribution of benthic-suspension feeders (Graf et al. 1982; Smaal et al. 1986; Gremare et al. 1997). Temperature and food availability have been proposed as the most important environmental factors affecting the dynamics of benthic invertebrates (Coma et al. 2000); however, competition for space, food abundance, and water movement are also likely to be important factors explaining seasonal patterns of growth, reproduction, and abundance of temperate benthic suspension-feeders (Boero et al. 1986; Coma et al. 2000). Studies of the seasonal patterns of the activity and secondary production of benthic suspension-feeders have been primarily conducted in cold temperate seas, and from these studies, an annual trend has emerged. The most characteristic aspects of these temporal patterns are winter dormancy and summer activity of organisms (Coma et al. 2000), with settlement and recruitment occurring during spring (Duckworth & Battershill 2001). Some of the most important suspension-feeding groups that are subjected to such seasonal variations include hydroids (Gili

et al. 1998), ascidians (Ribes et al. 1998), bivalves (Ahn et al. 2003; Cloern & Jassby 2008) and sponges (Turon et al. 1998; Duckworth & Battershill 2001). In addition, several studies have also demonstrated marked seasonal variation in the feeding activity of other abundant benthic suspension-feeders including holothurians, polychaetes and bryozoans (Barnes & Clarke 1995). In sponges, seasonal studies have focused on aspects of their reproductive biology (Witte et al. 1997), population dynamics, changes in environmental factors affecting sponge growth (Turon et al. 1998; Bell 2008), survival and metabolite biosynthesis (Duckworth et al. 2003; 2004); which will also be influenced by food consumption and energy extraction from the environment. As discussed in previous chapters (see Chapters 1 and 3), sponges are sessile suspension-feeders that acquire their food by filtering suspended particulate matter (planktonic particles) from the water column, thereby linking the pelagic and benthic environments. Because temporal patterns in the water column are strongly related to dominant environmental factors, the study of temporal fluctuations in the quality and quantity of the food supply to sponges is of great interest in order to elucidate if sponges can change their feeding strategies in response to temporal differences in food availability (Duckworth & Battershill 2001). Hence, investigating variation in the concentration of food particles over time and examining changes in the amount of food available, and therefore the proportion consumed, was the focus of the present chapter.

This chapter addresses four main questions related to the temporal variation of food particles and consumption of picoplankton by three sponge species: 1) does the amount and relative proportion of the picoplanktonic species composition in the water column change seasonally and inter-annually? 2) Are there any temporal differences in the proportions of the different types of picoplankton consumed by the study species? 3) Do sponges consume different proportions/amounts of picoplanktonic cells in relation to their concentration in the water column (if they change seasonally)? And 4) Does the amount of carbon gained by the sponges change temporally? In order to answer these questions and to determine whether there are changes in the retention efficiency of each species, a factorial ANOVA was used to test the effect of picoplankton and time of year on the retention efficiency of each

species. Similarly, the same analysis was used to test the effect of picoplankton and time of year on the number of cells present in the ambient water over the study period. Water samples were analysed over time to test the hypothesis that there are temporal changes in the amount of picoplankton available to sponges, which might result in changes in the amount of picoplanktonic organisms consumed by the sponges over time, or in the proportions of different picoplanktonic organisms. I also investigated the proportion of the picoplanktonic organisms consumed by sponges to determine if there is any relationship between the proportions of the organisms retained and the ambient cell concentration, and test whether or not food concentration is important to the proportions of the picoplanktonic organisms consumed by the sponges.

6.2 Methods

6.2.1 Study sites and species description

The study sites are located within the Taputeranga Marine Reserve on the south coast of Wellington in New Zealand (see Chapter 2 for more details). Three encrusting demosponges were studied; *Haliclona venustina* (Bergquist), *Strongylacidon* sp., and *Crella incrustans* (Carter, 1885). These species were chosen because they are very common and their well defined exhalant oscula reduce the risk of sampling error facilitating *in situ* sampling. Here, it is worth mentioning that in retrospect, it would have been a better idea to include these species in the multi-species survey (Chapter 4). However, we realised the importance of this at the time of water sample analysis when the thesis work was so far along the line that I could not change the sampling design any more. The study species *H. venustina* is often found on vertical rock walls, on the sides of channels, and boulders; it is yellow coloured, has big well defined oscula ($\sim 4-5$ mm diameter), and it can be seen with finger-like outgrowths from basal mass. *C. incrustans* is a very conspicuous encrusting species found on boulders, rock walls, and crevices; it has a bright orange-red colour and a smooth surface with raised and well defined oscula ($\sim 4-5$ mm diameter). *Strogylacidon* sp. is commonly found on boulders and rock walls; it has well defined oscula ($\sim 3-4$ mm dia-

meter) and has a smooth surface with a blue-grey colour (see species photos in Appendix A).

6.2.2 Sampling and data analysis

Sampling was carried out at three different times of each year in: winter (samples collected between June and August), spring (samples collected between September and November), and autumn (samples collected in March and April) of 2008, 2009 and 2010. Due to unfavourable/poor weather conditions that limit dive time in the study area, three sponge specimens from each species were used for this study. Methodological problems were encountered with some samples during collection and during the flow cytometry analyses; hence no summer data were available. Seawater samples for flow cytometry analysis were collected *in situ* by SCUBA divers following the sampling method described in previous chapters (see Chapters 2 and 3). After collection, water samples were fixed and prepared for flow cytometry analysis, and the flow cytometry analysis was carried out as explained in Chapter 2.

The efficiency of the sponges in retaining picoplanktonic particles (the retention efficiency), was calculated from the difference in concentrations between ambient (inhalant) and exhalant water samples as Equation 2.1:

The difference between cell concentrations in ambient and exhalant water samples calculated by flow cytometry, gave the number of cells removed (consumed) by the study species:

$$No.cellsremoved = Camb - Cexh \tag{6.1}$$

Here, it is important to clarify that the term removal or consumption is being defined as the amount of food that the sponge actually consumes. It refers to the amount of food that is available in the surrounding water, which then enters the inhalant canal of the sponge and is finally taken into its cells (i.e. the amount of carbon consumed by the sponge). The amount of carbon consumed by each cell type was calculated by multiplying the number of cells retained by the mean quantity of carbon contained in each cell according to its type, by using carbon conversions from the literature

as previously specified in Chapter 4. The percentage of carbon represented by each cell type retained by the study species was calculated according to Topçu et al. (2010) using the formula:

$$\%Qc(i) = \frac{Qc(i) * 100}{\sum Qc(i)} \tag{6.2}$$

Where $\%Qc(i)$ is the percentage of carbon represented by the ith cell type retained, $Qc(i)$ is the quantity of carbon represented by the ith cell type retained, and $\sum Qc(i)$ is the sum of all $Qc(i)$. This calculation gave the total quantity of carbon consumed by each of the sponges. These calculations were calculated for each replicate (specimen) from each species.

To examine if the retention efficiency of picoplanktonic organisms varied with time of year, a factorial ANOVA was conducted where each sponge species was analysed separately (see Table 6.1 for a description of the terms included). Since retention efficiency was expressed as percentage data, the ANOVA was conducted on arcsine-square root transformed data to meet assumptions of normality and equal variance. Likewise, to examine changes in the proportion of picoplanktonic organisms removed (consumed) by the study species over time, a factorial ANOVA was conducted and data were logarithm-transformed to meet the assumptions of parametric analysis when necessary (Table 6.1). The same analysis was performed to examine changes in the concentration of picoplanktonic cells over time, and the factorial model included the terms number of cells, picoplankton, time of year, and all interactions among these terms (Table 6.1). To determine if the amount of carbon gained by the sponges changed temporally, a factorial ANOVA was conducted and data were logarithm transformed to meet the assumptions when necessary (Table 6.1). The assumption of homogeneity of variances was examined using Bartlett's test ($P < 0.05$ in all cases). To examine if there was a relationship between the number of cells consumed and the number of cells present in the ambient water, a correlation analysis was conducted. To determine the significance of the correlation, the Pearson product-moment correlation coefficient (PMCC) analysis was used. All statistical analyses were performed by `R` ver. 2.10 (R Development 6 Core Team 2011).

Table 6.1: Attributes (and levels) used in the analysis. Variable type codes: C continuous, N nominal

Variable	Type	Terms (attributes)
Retention efficiency	C	arcsine-square root transformed data ($\arcsin\sqrt{x}$)
Picoplankton	N	3 attributes: 1) heterotrophic bacteria, 2) Prochlorococcus, 3) Synechococcus
Time of year	N	6 attributes: Autumn2009, Autumn2010, Spring2008, Spring2009, Winter2008, Winter2009
Ambient cells	C	logarithm transformed ($\log_e$)
Cells removed	C	logarithm transformed ($\log_e$)
Carbon gained	C	logarithm transformed ($\log_e$)

6.3 Results

6.3.1 Temporal variation of picoplankton removal

The diet composition of *C. incrustans*, *H. venustina* and *Strongylacidon* sp. included heterotrophic bacteria, *Prochlorococcus* and *Synechococcus* and these picoplanktonic organisms were found throughout the 2-yr cycle. Retention efficiency varied significantly among picoplanktonic organisms and with sampling periods for the three study species as indicated by the factorial ANOVA (Table 6.2). *C. incrustans* mainly retained heterotrophic bacteria during spring of the first year ($94-96\%$) and autumn of the second year ($61-83\%$), and showed a very low retention of these cells during winter of the first year ($1-10\%$); the retention of *Prochlorococcus* was higher during autumn of the first year ($81-93\%$) and *Synechococcus* were retained with a higher efficiency during winter of the first year ($88-90\%$) (Fig. 6.1, a). *H. venustina* mainly retained *Prochlorococcus* during autumn of the first year (97%) and heterotrophic bacteria during spring of the first year and winter of the second year ($95-98\%$, same range for both times of the year), with a lower retention in winter of the first year ($3-20\%$), whereas *Synechococcus* were retained with no more than 59% over the sampling period. No *Pro-*

chlorococcus were retained by this species during spring of the first year, but were retained in spring of the second year (Fig. 6.1, b). *Strongylacidon* sp. mainly retained *Prochlorococcus* during winter of the first year ($49-87\%$) and heterotrophic bacteria during spring of the first year ($64-80\%$). Contrastingly, this species had a very low retention of heterotrophic bacteria during winter of the first year ($2-5\%$) and no *Prochlorococcus* were retained by this species during spring of the first year, but were retained in spring of the second year with a low efficiency ($9-11\%$); *Synechococcus* were retained with a higher efficiency during autumn of the first year ($42-67\%$) and a very low retention was measured during spring of the first year ($3-15\%$) (Fig. 6.1, c).

Table 6.2: Factorial analysis of variance showing the interaction between picoplankton and time of year, on the retention efficiency of each of the study species. Significance at the 5% level.

Source of variation	df	*C. incrustans* $F\ value, P\ value$	*H. venustina* $F\ value, P\ value$	*Strongylacidon* sp. $F\ value, P\ value$
Picoplankton	2, 36	$21.151 < 0.001$	$0.788 < 0.001$	$1.656 < 0.001$
Time of year	5, 36	$20.726 < 0.001$	$9.816 < 0.001$	$4.943 < 0.001$
Picoplankton * Time of year	10, 36	$16.844 < 0.001$	$21.695 < 0.001$	$20.282 < 0.001$

6.3.2 Changes in picoplankton concentration over time

Ambient concentrations of picoplanktonic cells in the study area (as determined from ambient samples collected next to the study species) varied significantly over time, as indicated by the factorial ANOVA (Table 6.3). The analysis showed that the concentration of each picoplankton type varied significantly over the sampling periods. The overall average number of cells and sampling times indicated that heterotrophic bacteria were the most abundant cell type in the water column throughout the 2 yr period ($2.6\times10^6\pm2.7\times10^6$ cells ml^{-1}), followed by *Synechococcus* ($9.8\times10^3\pm6.4\times10^3$ cells ml^{-1}) and

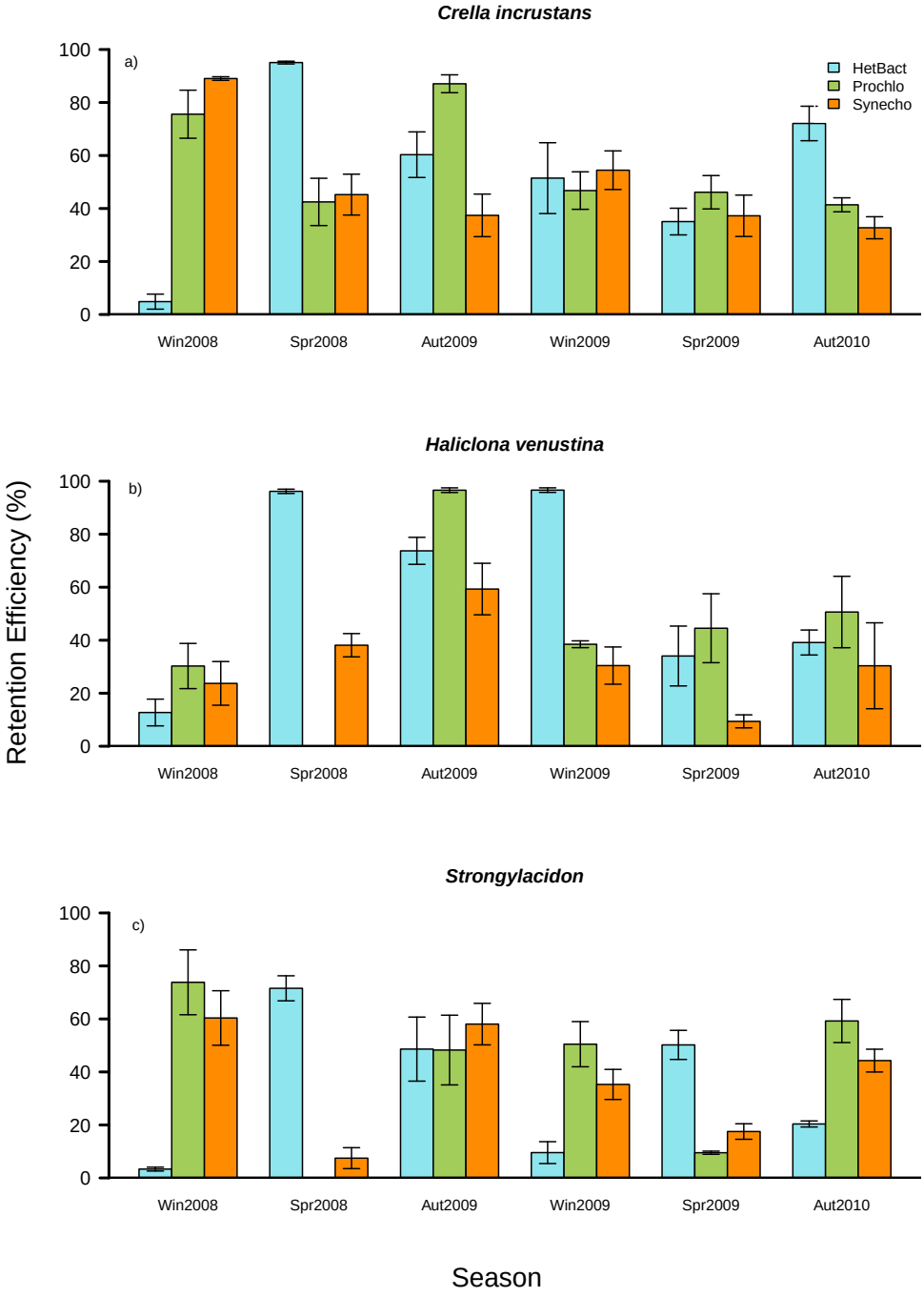


Figure 6.1: Retention efficiency (± StdDev) across sampling times during the 2-yr study period for a) *C. incrustans*, b) *H. venustina* and c) *Strongylacidon* sp. Retention efficiency is expressed as the percentage removal of heterotrophic bacteria (HetBAct), *Prochlorococcus* (Prochlo), and *Synechococcus* (Synecho) cells by the three study species as determined by the factorial ANOVA.

then *Prochlorococcus* ($9.0 \times 10^3 \pm 6.4 \times 10^3$ cells ml^{-1}). With respect to the time of year, heterotrophic bacteria were more abundant in spring of the second year ($4.8 \times 10^6 \pm 2.6 \times 10^6$ cells ml^{-1}), and the lowest concentration was observed in the autumn of the first year ($2.8 \times 10^5 \pm 1.8 \times 10^5$ cells ml^{-1}). *Synechococcus* were found in higher numbers during winter of the second year ($1.5 \times 10^4 \pm 9.1 \times 10^3$ cells ml^{-1}), and the lower concentration was observed in spring of the first year ($3.0 \times 10^3 \pm 9.5 \times 10^2$ cells ml^{-1}). *Prochlorococcus* showed higher numbers in winter of the first year ($1.5 \times 10^4 \pm 5.3 \times 10^3$ cells ml^{-1}) and a lower concentration was found in spring of the first year ($1.3 \times 10^2 \pm 1.4 \times 10^2$ cells ml^{-1}, $P < 0.001$) (Fig. 6.2).

Table 6.3: Factorial analysis of variance showing the effect of picoplankton and time of year on the number of cells present in the ambient water. Significance at the 5% level

Source of variation	df	Ambient cells $F\,value,\ P\,value$
Picoplankton	2, 144	$851.802 < 0.001$
Time of year	5, 144	$40.807 < 0.001$
Picoplankton * Time of year	10, 144	$21.991 < 0.001$

6.3.3 Temporal variation in the particles consumed by each species

The number of cells removed or consumed by the three study species varied significantly between the different types of picoplankton and with sampling periods, as indicated by the factorial ANOVA (Table 6.4). The three species showed a higher consumption of heterotrophic bacteria than the two types of cyanobacteria over the 2-yr period. *C. incrustans* consumed more heterotrophic bacteria during spring of both years ($1.7 \times 10^6 \pm 1.0 \times 10^5$ cells ml^{-1}, spring 2008 and $1.4 \times 10^6 \pm 3.4 \times 10^5$ cells ml^{-1}, spring 2009) and the lower consumption was found during winter and autumn of the first year ($9.8 \times 10^4 \pm 1.0 \times 10^5$ and $5.5 \times 10^4 \pm 3.1 \times 10^4$ cells ml^{-1}, respectively); more *Prochlorococcus* were consumed by this species during autumn of the first year ($1.5 \times 10^4 \pm 3.7 \times 10^3$ cells ml-1), and less during spring of the

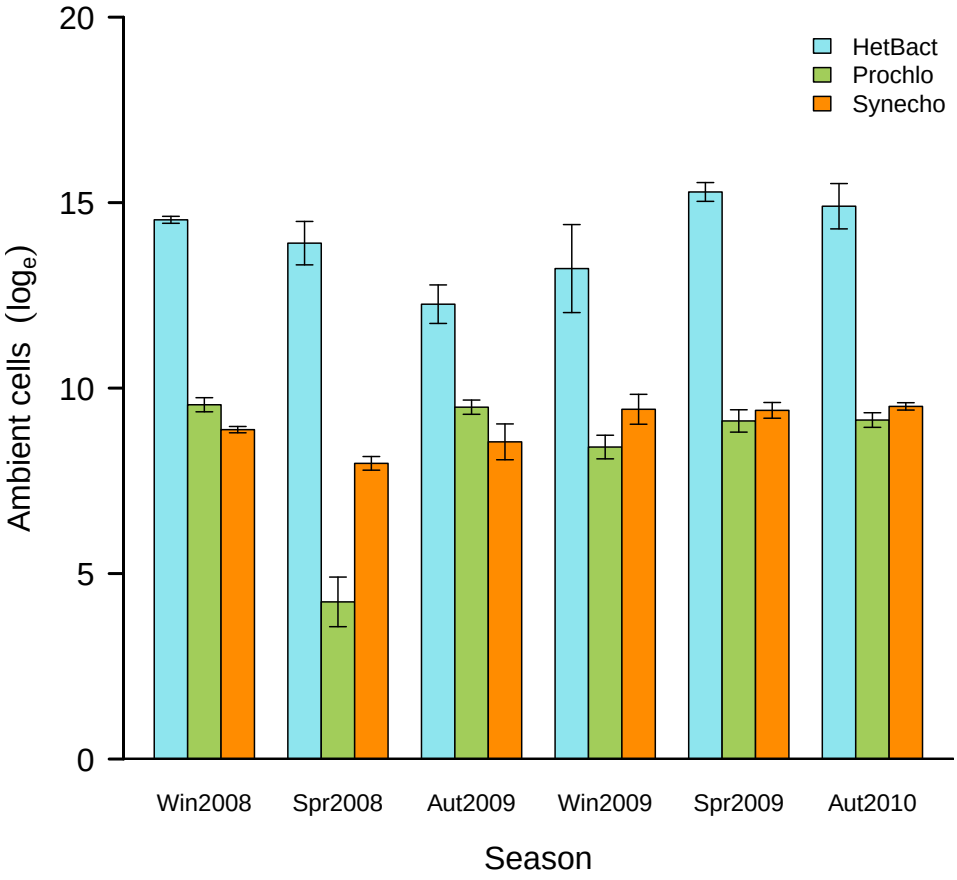


Figure 6.2: Ambient concentration of picoplankton (± StdDev) showing differences in the number of cells present in the water column over time. The number of cells was plotted in log scale in order to visually compare the concentrations of each type of picoplankton.

first year ($1.3 \times 102 \pm 4.1 \times 101$ cells ml^{-1}); this species consumed more *Synechococcus* in winter of the second year ($1.2 \times 10^4 \pm 3.6 \times 10^3$ cells ml^{-1}) and less in autumn of the first year ($1.1x103 \pm 9.7x102$ cells ml^{-1}) (Fig. 6.3, a). *H. venustina* consumed more heterotrophic bacteria in winter of the second year ($6.6 \times 10^6 \pm 4.0 \times 10^6$ cells ml^{-1}) and less in winter of the first year ($2.8 \times 10^5 \pm 2.2 \times 10^5$ cells ml^{-1}); this species consumed more *Prochlorococcus* in autumn of the first year ($1.5 \times 104 \pm 1.3 \times 103$ cells ml^{-1}) and less in spring of the first year ($3.6 \times 10^1 \pm 1.6 \times 10^1$ cells ml^{-1}); this species consumed more *Synechococcus* in winter of the second year ($4.9 \times 10^3 \pm 2.0 \times 10^3$ cells ml^{-1}) and less in spring of the second year ($1.1 \times 10^3 \pm 3.4 \times 10^2$ cells ml^{-1}) (Fig. 6.3, b). Finally, *Strongylacidon* sp. removed more heterotrophic bacteria in spring of the second year ($3.5 \times 10^6 \pm 2.3 \times 10^6$ cells ml^{-1}) and less in winter of the second year ($6.0 \times 10^3 \pm 5.5 \times 10^3$ cells ml^{-1}); more *Prochlorococcus* were consumed by this species during winter of the first year ($1.1 \times 10^4 \pm 5.3 \times 10^3$ cells ml^{-1}) and less during spring of the first year ($3.2 \times 10^1 \pm 7.8 \times 10^0$ cells ml^{-1}); this species consumed more *Synechococcus* in autumn of the first year ($6.4 \times 10^3 \pm 3.3 \times 10^3$ cells ml^{-1}) and less in spring of the first year ($1.8 \times 10^2 \pm 1.9 \times 10^2$ cells ml^{-1}) (Fig. 6.3, c).

Table 6.4: Factorial analysis of variance showing the interaction between picoplankton and time of year, on the number of cells removed by each of the study species. Significance at the 5% level

Source of variation	df	*C. incrustans* $F\,value, P\,value$	*H. venustina* $F\,value, P\,value$	*Strongylacidon* sp. $F\,value, P\,value$
Picoplankton	2, 36	$21.151 < 0.001$	$0.788 < 0.001$	$1.656 < 0.001$
Time of year	5, 36	$20.726 < 0.001$	$9.816 < 0.001$	$4.943 < 0.001$
Picoplankton * Time of year	10, 36	$16.844 < 0.001$	$21.695 < 0.001$	$20.282 < 0.001$

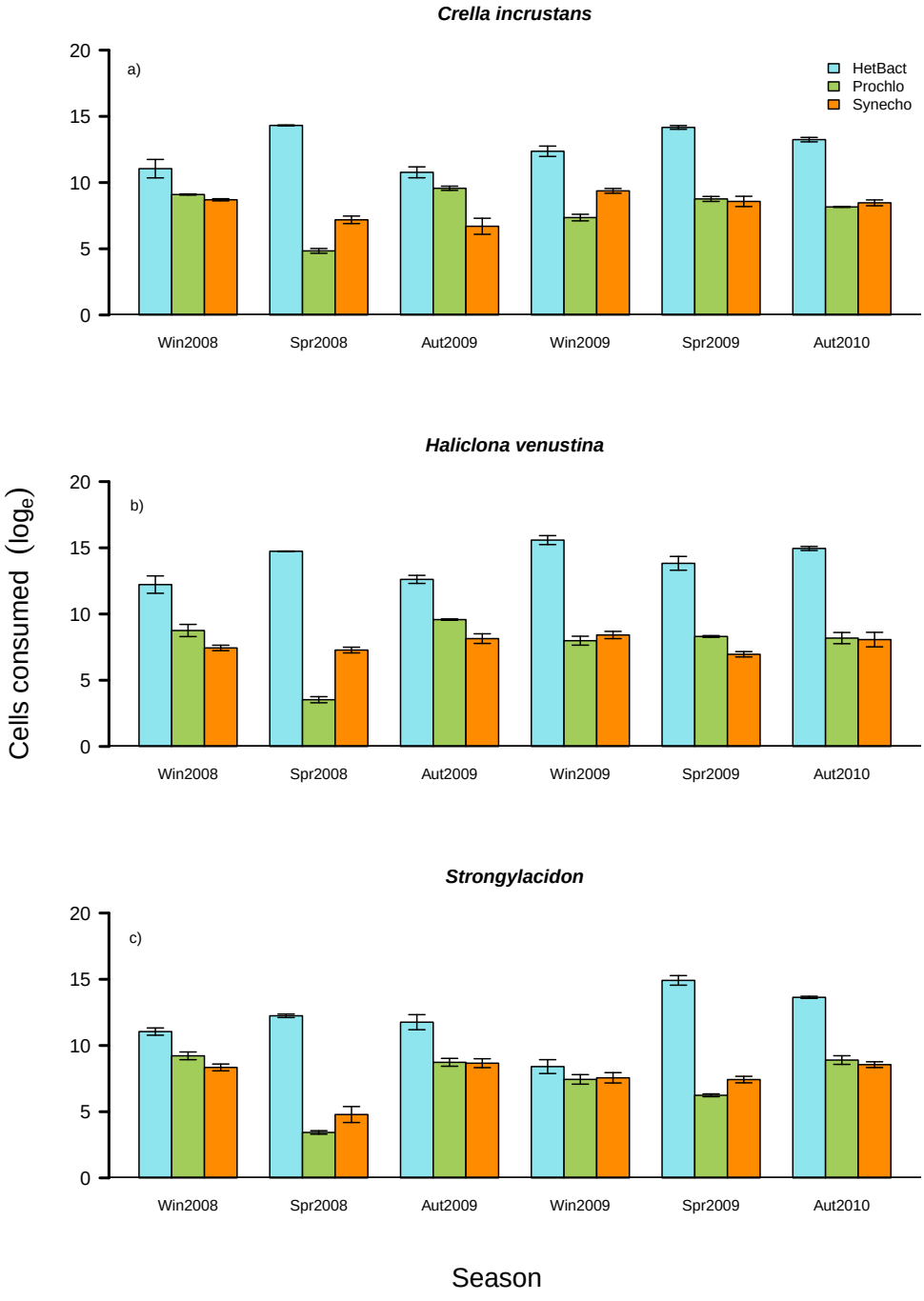


Figure 6.3: Number of cells consumed of each type of picoplankton by the three study species over time: a) *C. incrustans*, b) *H. venustina* and c) *Strongylacidon* sp. The number of cells consumed was plotted in log scale in order to visually compare the concentrations of each type of picoplankton. Data presented are averages (± StdDev).

6.3.4 Correlation between the abundance and removal of picoplankton

A correlation analysis was conducted to determine if sponges consumed different proportions of picoplanktonic cells in relation to their concentration in the water column. This analysis was performed considering an overall average removal and abundance of picoplankton over the study period, including the three food types and the three sampling times/seasons. The results indicate a significant correlation between the number of cells removed by *C. incrustans* ($r^2 = 0.96$; $P < 0.001$), *H. venustina* ($r^2 = 0.97$; $P < 0.001$) and *Strongylacidon* sp. ($r^2 = 0.94$; $P < 0.001$), and the abundance of cells in the ambient water (Fig. 6.4).

6.3.5 Amount of carbon gained by the sponges over time

The amount of carbon gained by the three study species varied significantly among each type of picoplankton and with sampling periods, as indicated by the factorial ANOVA (Table 6.5). Heterotrophic bacteria constituted the most important carbon source, and most of the carbon was supplied by this type of picoplankton for the three species throughout the study period. On an annual basis, consumption of heterotrophic bacteria provided the study species with 809.4 mg C y^{-1}, while consumption of *Prochlorococcus* and *Synechococcus* provided with 10.4 and 21.8 mg C y^{-1}, respectively. In terms of picoplanktonic biomass, the major part of the carbon consumed by *C. incrustans* in winter originated from heterotrophic bacteria constituting 65% of the carbon retained by the sponge, while 29% was supplied from *Synechococcus* and 6% from *Prochlorococcus*. During spring most of the carbon was also supplied by heterotrophic bacteria and constituted 97% of the total carbon retained with only 2% and 1% supplied from *Synechococcus* and *Prochlorococcus*, respectively. In autumn most of the carbon was supplied by heterotrophic bacteria representing 85% of the total carbon retained by the sponge. For *H. venustina*, the major part of the carbon retained in winter and spring originated from heterotrophic bacteria representing 99% with only 1% supplied by *Synechococcus*; in autumn most of the carbon was supplied by heterotrophic bacteria and constituted 96% with 2% supplied by each type of cyanobacteria. For *Strongylacidon* sp., heterotrophic bacteria sup-

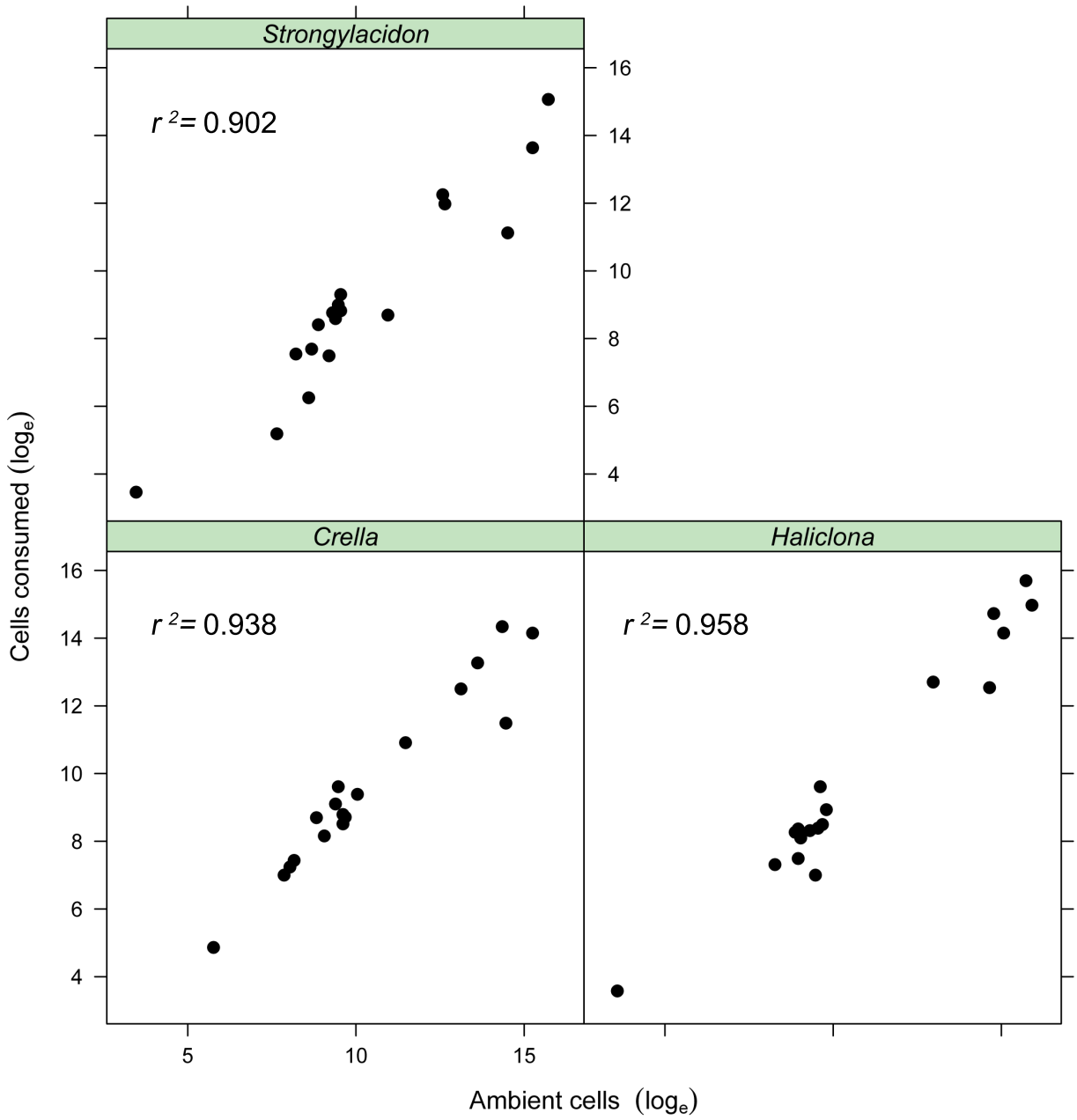


Figure 6.4: Relationship of the logarithmically converted number of cells removed (vertical axis) and the number of cells present in the ambient water (horizontal axis). Data used for the correlation analysis includes the three types of picoplankton present in the ambient water and the number of cells removed by each species in each of the sampling times over the 2-yr period.

plied 43% of the total carbon retained in winter with 34% and 23% supplied from *Synechococcus* and *Prochlorococcus*, respectively. In spring 99.5% of the total carbon retained was supplied by heterotrophic bacteria, and in autumn 87% was supplied by heterotrophic bacteria with 9% and 4% supplied from *Synechococcus* and *Prochlorococcus*, respectively (Fig. 6.5).

Table 6.5: Factorial analysis of variance showing the interaction between picoplankton and time of year, on the amount of carbon gained by each of the study species. Significance at the 5% level.

Source of variation	df	*C. incrustans* $F\,value, P\,value$	*H. venustina* $F\,value, P\,value$	*Strongylacidon* sp. $F\,value, P\,value$
Picoplankton	2, 45	$43.666 < 0.001$	$75.947 < 0.001$	$39.424 < 0.001$
Time of year	2, 45	0.1226 0.8849	$3.8650 < 0.05$	$11.372 < 0.001$
Picoplankton * Time of year	4, 45	$8.0023 < 0.001$	$2.9293 < 0.05$	$15.462 < 0.001$

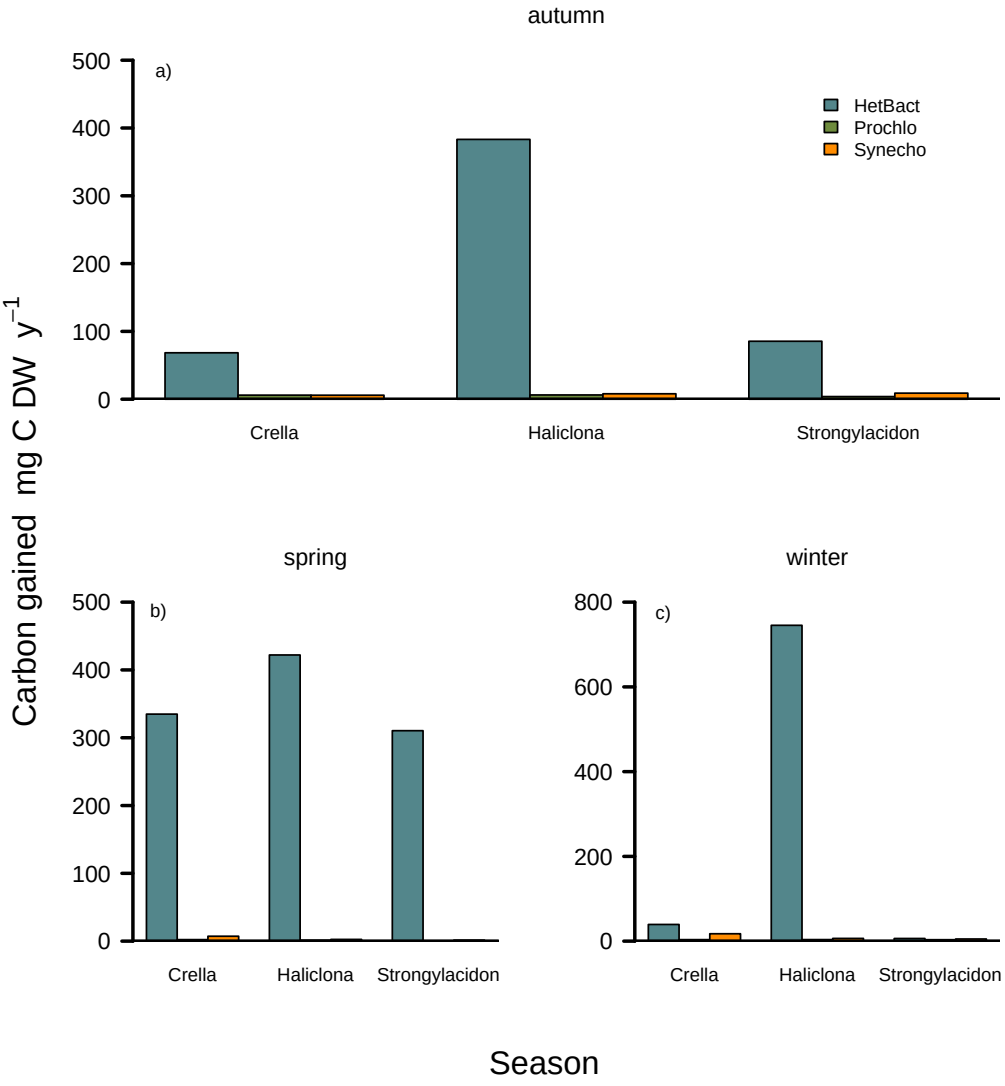


Figure 6.5: Average amount of carbon (mg C DW y^{-1}) consumed by the three study species during the sampling times of autumn, spring and winter over the study period. The bars represent each of the picoplanktonic organisms that sponges removed from the water column over different times of the year.

6.4 Discussion

6.4.1 Temporal variation of picoplankton in the water column

The amount of food (picoplankton) present in the water column varied significantly over the 2-yr study period. The samples collected from the surrounding water of the three study species, revealed significant differences in the concentrations of picoplankton at the different time intervals. The results showed that on average, heterotrophic bacteria ($1.2 \times 10^7 \pm 9.6 \times 10^6$ cells ml^{-1}) and *Synechococcus* ($4.2 \times 10^4 \pm 1.7 \times 10^4$ cells ml^{-1}) were more abundant throughout the second year, and *Prochlorococcus* ($2.9 \times 10^4 \pm 9.3 \times 10^3$ cells ml^{-1}) were more abundant in the first year. In terms of interannual differences, heterotrophic bacteria showed stronger annual variation in the concentrations of each of the picoplanktonic organisms in the sampling times (autumn, spring and winter) between both years. Contrastingly, *Prochlorococcus* and *Synechococcus* varied significantly between spring of both years. It is important to note that the quality and quantity of organic matter vary both temporally and spatially in response to physical and biological factors, and depend on the intensity of the primary production in the water column (Danovaro & Fabiano 1997). Events such as La Niña or El Niño have different impacts on New Zealand's climate. The last La Niña event was in 2007/08 (NIWA), so it is possible that water temperature and wind patterns may also have an effect on the abundance of picoplankton in the study area. These factors may have affected the abundance of cyanobacteria in the water column of spring of the first year, resulting in lower numbers of *Prochlorococcus* at that time of the year. It is worth mentioning that the conclusions presented here about the temporal variation of picoplankton in the study area, are being drawn based on six sampling occasions over a 2-year study period. Although temporal studies usually yield more conclusive evidence when monitored year-round and in the long term (> 5 years), the results presented here provide an important contribution to the knowledge of bacterioplankton concentration in the water column, with the time frame available to carry out this research.

6.4.2 Do sponges consume picoplanktonic particles in relation to their abundance in the water column?

In terms of the number of cells consumed, heterotrophic bacteria represented the most important group, followed by *Prochlorococcus* and then *Synechococcus*. The removal (consumption) of food particles by the sponge species also varied significantly over time. For the three species, heterotrophic bacteria represented 79 to 93% of the total cells consumed in winter, spring and autumn, respectively, whereas *Prochlorococcus* and *Synechococcus* represented only 0.5 to 14% of the cells removed at the same time periods over the 2-yr period. It is interesting that the number of cells removed by the three species across the sampling periods were consistent with temporal fluctuations in resource (food) abundance. The PMCC analysis was used to determine if these changes in number of cells removed were the result of the declines or increases in the different picoplanktonic groups available in the water column. A significant correlation was found in most cases; the only exceptions were *C. incrustans* and *H. venustina* where no significant correlation was found between the number of heterotrophic bacteria and *Synechococcus* consumed in relation to their abundance in the water column. However, when the correlation analysis was performed considering an overall average removal and abundance of picoplankton over the study period (including the three food types and the three times of year/seasons), the results indicate a significant correlation between the number of cells removed by *C. incrustans* (r^2= 0.96; $P < 0.001$), *H. venustina* ($r^2 = 0.97$; $P < 0.001$) and *Strongylacidon* sp. ($r^2 = 0.94$; $P < 0.001$), and the abundance of cells in the ambient water.

The hypothesis that sponges exploit the food particles that are more abundant in the water column could not be tested in the one-off, multi-species study described in Chapter 4 (as there was not enough variation in the food concentrations). However, in the current chapter the hypothesis that the consumption of planktonic particles is proportional to the concentration or availability of food particles was tested using the results obtained from this 2-yr study. Coma et al. (2001) showed for the first time, that sessile suspension-feeders consumed a broad spectrum of planktonic particles appropriate to each filtering mechanism in proportion to their abundance in

the water column. In the sponge species studied here, such a positive relation between particle concentration and retention efficiency was observed for heterotrophic bacteria, suggesting that the study species seemed to exploit the most abundant food-type (heterotrophic bacteria) in the ambient water. The three types of picoplankton were retained with different efficiencies by the sponge species over the 2-yr period. On an annual basis, the three species had high retention rates of *Prochlorococcus* during autumn (64%) and winter (53%); but high retention of heterotrophic bacteria during spring (64%). These results demonstrated that the study species removed both prokaryotic and cyanobacterial cells, but with varying efficiencies. *C. incrustans* showed a higher retention efficiency of *Prochlorococcus* all through the study period; in contrast, *H. venustina* showed a higher retention for heterotrophic bacteria, and *Strongylacidon* sp. had a higher retention efficiency of *Synechococcus* throughout the study period. These differences in uptake or retention of picoplanktonic cell types may be explained by capture mechanisms and particle digestibility (Topcu et al. 2010), variation in choanocyte numbers and physiological mechanisms of feeding (Ley & Eerkes-Medrano 2006), and complexity of aquiferous systems (Weisz et al. 2008). Yahel et al. (2006) examined the mechanisms involved in the selective retention of particles in hexactinellid glass sponges and concluded that the selective retention observed involved individual processing, recognition, sorting and transport of each particle through the sponge's syncytial tissue. However, the exact mechanism by which sponges are able to select food particles is uncertain.

The term retention rate (or retention efficiency) refers to the rate at which particles are retained from the water pumped through the sponge's aquiferous system (Turon et al. 1997). Although selectivity is often understood as an active choice for different types of particles, in the sponge literature, particle uptake is associated with the preference (also termed selectivity) for certain particles (e.g. Yahel et al. 200; Hanson et al. 2009; Topçu et al. 2010). Several studies have shown different particle retention efficiencies between co-occurring species (Pile et al. 1997; Kowalke 2000; Pile 2005), suggesting that particle selectivity depends on the size of the particles filtered (Van De Vyver et al. 1990; Turon et al. 1997) and that both particle concentra-

tion and size affect retention ability (Huysecom et al. 1988; Duckworth & Battershill 2001). In previous chapters I discussed that *Synechococcus* and *Prochlorococcus* are larger than heterotrophic bacteria, and in Chapter 3 the preference for different particle sizes by different sponge species was discussed concluding that for the seven species studied, particle size was an important factor influencing the retention of food particles given that five of the sponges retained bigger cells (cyanobacteria) with a high efficiency and two sponge species had high retention of smaller cells (heterotrophic bacteria). It is possible that the cell size can be a plausible explanation for the different retention efficiencies observed for the study species in this chapter. The results from my study suggest that the smaller food particles were retained with higher efficiency during spring (overall average across species, 64%), and bigger cells (*Prochlorococcus*) were retained with higher efficiency during autumn and winter (overall average across species 64% and 53%, respectively) over the 2-yr period, showing the ability of the study species to select particles. Active selection based on particle size has already been demonstrated by other authors (Ribes et al. 1999a; Topçu et al. 2010), and in a recent *in situ* study of the sponge *Callyspongia* sp. from the west coast of Australia, Hanson et al. (2009) showed an overall preference for *Synechococcus* over heterotrophic bacteria suggesting that cell size is an important factor in the differential uptake of the two types of picoplankton identified in their study. In support of this concept, a study looking at particle uptake by a calcareous sponge showed that the mechanism of food uptake at the cellular level varied with the size and type of particles (Leys & Eerkes-Medrano 2006).

6.4.3 Can sponges select food particles that optimise their nutritional intake?

In Chapter 4, I discussed the premise that sponges are responsible for a large proportion of the energy flow from the plankton to the benthos. The results from the present chapter indicate that the study species feed more efficiently on smaller cells (heterotrophic bacteria) than on bigger cells (*Prochlorococcus* and *Synechococcus*), and that the higher concentration in the water column of heterotrophic bacteria meant that they contributed the most to sponge

diets with a greater carbon uptake. This was corroborated from calculations of overall carbon consumed by the study species at different time intervals (winter, spring, summer) over a two-year period. The proportions of the picoplanktonic organisms retained by the sponge species changed between species and over time, and the main source of carbon for the three species came from heterotrophic bacteria. The high abundance of heterotrophic bacteria in the ambient water throughout sampling periods and over the two-year study period, may explain why these picoplanktonic cells comprised a major food source for the three species studied. In this study, heterotrophic bacteria were the most abundant food source throughout the 2-yr study period and represented the main source of carbon for the study species. These results are in agreement with previous studies where heterotrophic bacteria have been found to be one of the primary sources of carbon for sponges (Ribes et al. 1999b; Trussell et al. 2006; Yahel et al. 2007).

In general, the three species studied here consumed the different food particles throughout most of the study period except for *H. venustina* and *Strongylacidon* sp. that did not retain *Prochlorococcus* during spring of the first year, which corresponded with a period of lower abundance of this cell-type in the water column. Environmental factors such as temperature, salinity and water movement can influence food consumption (Bastolla et al. 2005; Tsounis et al. 2006), and previous studies have suggested that temperature and nutrient availability influence the abundance and distribution of *Prochlorococcus* (Partensky et al. 1999; Bertilsson et al. 2003). As previously discussed, the removal of food particles was found to be influenced by the concentration of particles available in the water column. So, it is possible that environmental conditions affected the biomass of *Prochlorococcus* during spring of the first year when the lower numbers of these cells where registered; and therefore, influenced the removal of these particles by *H. venustina* and *Strongylacidon* sp.

There is a predominant pattern of growth and reproduction of sponges during spring and summer (Garrabou & Zabala 2001; Coma & Ribes 2003; Bell 2008). In previous studies, the observed differences among months appear to be consistent with the demands of seasonal cycles in growth and reproduction, suggesting that endogenous metabolic demands are an im-

portant factor determining feeding behaviour of temperate sponges (Barthel 1986; Ribes et al. 2003). The relatively constant retention and consumption of food particles throughout the year, together with a lack of any temporal trend in cell concentrations and energy (carbon) intake, suggests that the dynamics of the sponge species studied here do not appear to be subjected to seasonal patterns. However, it remains to be elucidated if sponges exhibit different growth and production rates as a result of differences in food quality and quantity over annual cycles in the study area. The ability of sponges to efficiently capture picoplankton (particles smaller than 2 µm) has been comprehensively discussed throughout this thesis. However, there is also evidence that sponges are able to capture larger cells (up to 50 µm) such as phytoplankton (Reiswig 1971b; Frost 1981; Yahel et al. 1998), and Ribes et al. (1999a) found that the contribution of such large cells in the energy budget of the sponge *Dysidea avara* varied seasonally, following the planktonic composition of the water column. In this study, the presence of larger food particles ($2 - 10$ µm) was not assessed but it is worth considering that the availability of larger particles in the water column might vary seasonally, thus influencing consumption of other smaller particles.

To conclude, the results indicate that there is a wide range of food concentrations in the rocky reefs where the study species are living, over which retention rate of sponges varies temporally. This emphasises the importance of understanding temporal variations in productivity, and suggests that such variations are likely to have important implications for suspension-feeders. The sponge species analysed here seem to exhibit different feeding behaviours at varying food concentrations as well as different retention rates across sampling periods; indicating that consumption is proportional to availability, and sponges exploit the most abundant food particles in the water column and also select the food particles that optimise their nutritional intake. Finally, an *in situ* study such as this, with little control over either food or temperature effects on sponges, cannot fully explain the interactions between feeding, food availability and energy demand. However, the findings from this study suggest that the dynamics of the sponge species throughout the year do not appear to be related to food availability, although it remains to be elucidated if changes in food quality and quantity over temporal scales

have an effect on biological processes of sponges in the temperate rocky reef studied. In the next and final chapter, the temporal results from this study regarding the consumption of carbon by the study species from the pico-planktonic particles removed, will be integrated into a preliminary carbon budget where I will infer the potential for the carbon consumed to support sponge metabolism as well as other processes such as sponge growth and reproduction.

6.5 References

— Ahn I.Y., Surh J., Park Y.G., Kwon H., Choi K.S., Kang S.H., Choi H.J., Kim K.W. & Chung H. (2003). Growth and seasonal energetics of the Antarctic bivalve *Laternula elliptica* from King George Island, Antarctica. Marine Ecology-Progress Series, 257, 99-110.

— Barthel D. (1986). On the ecophysiology of the sponge *Halichondria panicea* in Kiel Bight .1. Substrate-specificity, growth and reproduction. Marine Ecology-Progress Series, 32, 291-298.

— Bavestrello G., Puce S., Cerrano C., Zocchi E. & Boero F. (2006). The problem of seasonality of benthic hydroids in temperate waters. Chemistry and Ecology, 22, 197-205.

— Bell J.J. (2008). Sponges as agents of biological disturbance. Marine Ecology-Progress Series, 368, 127-135.

— Bertilsson S., Berglund O., Karl D.M. & Chisholm S.W. (2003). Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea. Limnology and Oceanography, 48, 1721-1731.

— Beukema J.J. & Cadee G.C. (1991). Growth rates of the bivalve *Macoma balthica* in the Wadden Sea during a period of eutrophication - relationships with concentrations of pelagic diatoms and flagellates. Marine Ecology-Progress Series, 68, 249-256.

— Boero F., Balduzzi A., Bavestrello G., Caffa B. & Vietti R.C. (1986). Population-dymanics of *Eudendrium glomeratum* (Cnidaria, Anthomedusae) on the Portofino Promontory (Ligurian sea). Marine Biology, 92, 81-85.

— Boero F., Belmonte G., Fanelli G., Piraino S. & Rubino F. (1996). The continuity of living matter and the discontinuities of its constituents: Do plankton and benthos really exist? Trends in Ecology & Evolution, 11, 177-180.

— Cadee G.C. (1986). Recurrent and changing seasonal patterns in phytoplankton of the westernmost inlet of the Dutch Wadden Sea from 1969 to 1985. Marine Biology, 93, 281-289.

— Cloern J.E. (1996). Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. Reviews of Geophysics, 34, 127-168.

— Cloern J.E. & Jassby A.D. (2008). Complex seasonal patterns of primary producers at the land-sea interface. Ecology Letters, 11, 1294-1303.

— Coma R. & Ribes M. (2003). Seasonal energetic constraints in Mediterranean benthic suspension feeders: effects at different levels of ecological organization. Oikos, 101, 205-215.

— Coma R., Ribes M., Gili J.M. & Hughes R.N. (2001). The ultimate opportunists: consumers of seston. Marine Ecology-Progress Series, 219, 305-308.

— Coma R., Ribes M., Gili J.M. & Zabala M. (2000). Seasonality in coastal benthic ecosystems. Trends in Ecology & Evolution, 15, 448-453.

— Danovaro R. & Fabiano M. (1997). Seasonal changes in quality and quantity of food available for benthic suspension-feeders in the Golfo Marconi (north-western Mediterranean). Estuarine Coastal and Shelf Science, 44, 723-736.

— Duckworth A.R. & Battershill C.N. (2001). Population dynamics and chemical ecology of New Zealand demospongiae *Latrunculia* sp nov and *Polymastia croceus* (Poecilosclerida : Latrunculiidae : Polymastiidae). New Zealand Journal of Marine and Freshwater Research, 35, 935-949.

— Frost T.M. (1981). Analysis of ingested particles within a fresh-water sponge. Transactions of the American Microscopical Society, 100, 271-277.

— Gili J.M., Alva V., Coma R., Orejas C., Pages F., Ribes M., Zabala M., Arntz W., Bouillon J., Boero F. & Hughes R.G. (1998). The impact of small benthic passive suspension feeders in shallow marine ecosystems: The hydroids as an example. Zoologische Verhandelingen (Leiden), 0, 99-105.

— Graf G. (1989). Benthic pelagic coupling in a deep-sea benthic community. Nature, 341, 437-439.

— Graf G., Bengtsson W., Diesner U., Schulz R. & Theede H. (1982). Benthic response to sedimentation of a spring phyoplankton bloom - process and budget. Marine Biology, 67, 201-208.

— Gremare A., Amouroux J.M., Charles F., Dinet A., RiauxGobin C., Baudart J., Medernach L., Bodiou J.Y., Vetion G., Colomines J.C. & Albert P. (1997). Temporal changes in the biochemical composition and nutritional value of the particulate organic matter available to surface deposit-feeders: A two year study. Marine Ecology-Progress Series, 150, 195-206.

— Hanson C.E., McLaughlin M.J., Hyndes G.A. & Strzelecki J. (2009). Selective uptake of prokaryotic picoplankton by a marine sponge (*Callyspongia* sp.) within an oligotrophic coastal system. Estuarine Coastal and Shelf Science, 84, 289-297.

— Helson J.G., Pledger S. & Gardner J.P.A. (2007). Does differential particulate food supply explain the presence of mussels in Wellington Harbour (New Zealand) and their absence on neighbouring Cook Strait shores? Estuarine Coastal and Shelf Science, 72, 223-234.

— Huysecom J., Richelle-Maurer E., Vyver G.V.D. & Vray B. (1988). Effect of bacterial concentration on retention and growth rate of the freshwater sponge *Ephydatia fluviatilis*. Physiological Zoology, 61, 535-542.

— Kowalke J. (2000). Ecology and energetics of two Antarctic sponges. Journal of Experimental Marine Biology and Ecology, 247, 85-97.

— Leys S.P. & Eerkes-Medrano D.I. (2006). Feeding in a calcareous sponge: Particle uptake by pseudopodia. Biology Bulletin, 211, 157-171.

— NIWA. National Institute of Water and Atmospheric Research. URL http://www.niwa.co.nz/education-and-training/schools/students/enln

— Partensky F., Hess W.R. & Vaulot D. (1999). *Prochlorococcus*, a Marine Photosynthetic Prokaryote of Global Significance. Microbiology and Molecular Biology Reviews, 63, 106-127.

— Pile A.J. (2005). Overlap in diet between co-occurring active suspension feeders on tropical and temperate reefs. Bulletin of Marine Science, 76, 743-749.

— Pile A.J., Patterson M.R., Savarese M., Chernykh V.I. & Fialkov V.A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnology and Oceanography, 42, 178-184.

— Reiswig H.M. (1971b). Particle feeding in natural population of 3 marine demosponges. Biological Bulletin, 141, 568-591.

— Ribes M., Coma R. & Gili J.M. (1998). Seasonal variation of *in situ* feeding rates by the temperate ascidian *Halocynthia papillosa*. Marine Ecology-Progress Series, 175, 201-213.

— Ribes M., Coma R. & Gili J.M. (1999a). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Ribes M., Coma R. & Gill J.M. (1999b). Seasonal variation of particulate organic carbon, dissolved organic carbon and the contribution of microbial communities to the live particulate organic carbon in a shallow near-bottom ecosystem at the Northwestern Mediterranean Sea. Journal of Plankton Research, 21, 1077-1100.

— Ribes M., Coma R. & Rossi S. (2003). Natural feeding of the temperate asymbiotic octocoral-gorgonian *Leptogorgia sarmentosa* (Cnidaria : Octocorallia). Marine Ecology-Progress Series, 254, 141-150.

— Smaal A.C., Verhagen J.H.G., Coosen J. & Haas H.A. (1986). Interaction between seston quantity and quality and benthic suspension feeders in the Oosterschelde, The Netherlands. Ophelia, 26, 385-399.

— Topçu N.E., Perez T., Gregori G. & Harmelin-Vivien M. (2010). *In situ* investigation of *Spongia officinalis* (Demospongiae) particle feeding: Coupling flow cytometry and stable isotope analysis. Journal of Experimental Marine Biology and Ecology, 389, 61-69.

— Trussell G.C., Lesser M.P., Patterson M.R. & Genovese S.J. (2006). Depth-specific differences in growth of the reef sponge *Callyspongia vaginalis*: role of bottom-up effects. Marine Ecology-Progress Series, 323, 149-158.

— Turon X., Galera J. & Uriz M.J. (1997). Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. Journal of Experimental Zoology, 278, 22-36.

— Turon X., Tarjuelo I. & Uriz M.J. (1998). Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defence. Functional Ecology, 12, 631-639.

— Van De Vyver G., Vray B., Belaouane S. & Toussaint D. (eds.)

— (1990). Efficiency and selectivity of microorganism retention by *Ephydatia fluviatilis*. Smithsonian Institution Press, Washington, DC, USA.

— Weisz J.B., Lindquist N. & Martens C.S. (2008). Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? Oecologia, 155, 367-376.

— Witte U., Brattegard T., Graf G. & Springer B. (1997). Particle capture and deposition by deep sea sponges from the Norwegian-Greenland Sea. Marine Ecology Progress Series, 154, 241-252.

— Yahel G., Eerkes-Medrano D.I. & Leys S.P. (2006). Size independent selective filtration of ultraplankton by hexactinellid glass sponges. Aquatic Microbial Ecology, 45, 181-194.

— Yahel G., Post A.F., Fabricius K., Marie D., Vaulot D. & Genin A. (1998). Phytoplankton distribution and grazing near coral reefs. Limnology and Oceanography, 43, 551-563.

— Yahel G., Whitney F., Reiswig H.M., Eerkes-Medrano D.I. & Leys S.P. (2007). *In situ* feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. Limnology and Oceanography, 52, 428-440.

Chapter 7

General discussion: The importance of picoplanktonic feeding to temperate sponges, and their ecological significance

7.1 Thesis summary

One major topic that has been discussed throughout this thesis is the potential for suspension-feeders to affect water column dynamics (i.e. the transportation of particulate organic matter, planktonic organisms and detritus from pelagic to benthic environments) as a result of their feeding activities. As mentioned in previous chapters, sponges are able to retain particles in the size range of $0.8 - 50$ µm with varying efficiencies. The sponge species studied here were found to feed mainly on picoplankton. Here it is worth mentioning that only in a few ambient samples, the fluorescence emission of a small percentage ($\sim 0.4 - 7\%$) of larger cells ($\sim$ 5µm) was detected; hence these cells were not included in any further analysis. Marine cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* comprise the prokaryotic component of the oxygenic photosynthetic picoplankton (Heldal et al. 2003) and were found in the water samples collected on the south coast of Wellington throughout the study period. These microscopic cells contribute around

half of the carbon fixed in marine systems and hence are of particular ecological significance with regard to global carbon cycling (Partensky et al. 1999). At my study sites, where there is a high diversity and abundance of sponges, I was interested in investigating the amount of the available picoplanktonic particles that sponges were removing, as well as how much carbon was supplied to sponges from these cells. Furthermore, I was also interested to determine if there was any differential utilisation of picoplankton that enables co-existence of different sponge species in this habitat.

The diet of the sponge species analysed in this thesis was comprised of three types of picoplanktonic organisms: heterotrophic bacteria, *Prochlorococcus*, and *Synechococcus*. In Chapter 2, I found that two species of calcareous sponges removed *Prochlorococcus* and *Synechococcus* with a higher efficiency than heterotrophic bacteria. This study was the first to investigate the natural diet of calcareous sponges in a temperate rocky subtidal reef using flow cytometry and provided evidence for differences in the diets of calcareous sponges and demosponges. In order to corroborate if such differences could be detected by analysing dietary differences, I included the two species of calcareous sponges with five demosponges in Chapter 3, where the preference for different particle sizes by different sponge species was discussed. One of the main conclusions was that for the seven species studied, particle size was an important factor influencing the retention of food particles given that five of the sponges retained bigger cells (cyanobacteria) with a high efficiency and two sponge species had high retention of smaller cells (heterotrophic bacteria). The differences in retention of the three cell types suggest that the study species prefer some food particles over others, and I proposed that niche partitioning is a potential mechanism that enables co-existence of different sponge species in this habitat. Furthermore, the results from this study raised questions about whether or not fewer larger cells (*Prochlorococcus* and *Synechococcus*) were able to provide the same amount of energy required to meet the sponges' metabolic demands as smaller, more abundant cells (heterotrophic bacteria), when retained with a high efficiency.

Correspondingly, the results from Chapters 4 and 6, indicated that the study species fed more efficiently on smaller cells (heterotrophic bacteria) than on bigger cells (*Prochlorococcus* and *Synechococcus*), and that higher

concentrations of heterotrophic bacteria in the water column meant that they contributed the most to sponge diets with a greater carbon uptake. It's been suggested that sponges, due to their high abundance, high filtration capacity, and a heterogeneous diet, may be important to nutrient dynamics in coastal ecosystems. In Chapter 4, I showed that sponge assemblages on a subtidal rocky reef, are capable of consuming a considerable proportion of the available picoplankton, and are therefore responsible for the transfer of large amounts of carbon from the water column to the benthos, so demonstrating the potential importance of sponges to energy flow in this ecosystem. It's been suggested that sponges, due to their high abundance, high filtration capacity, and a heterogeneous diet, may be important to nutrient dynamics in coastal ecosystems (Richter 2001; Ribes et al. 2005). Because throughout this thesis I found that sponges can efficiently filter and consume organic particles (picoplankton) from the water column, in Chapter 5 I investigated if sponges also increase the concentration of nutrients in the water column, and if the importance of sponges could be related to nutrient dynamics, their effect on water column nutrient concentrations and therefore ecosystem functioning. The results from this chapter showed that water column nutrient concentrations varied spatially and temporally; with the higher concentrations of ammonium and phosphate found during autumn, and the higher concentrations of nitrate found during winter. As a result of sponge feeding and metabolism of particles, sponges excrete dissolved inorganic and organic waste back into the water column, and thus are major contributors to the cycling of essential elements.

7.2 Carbon budgets

Although suspension-feeding is considered to be one of the most widespread feeding strategies among benthic organisms, the natural feeding ecology and energetics of benthic suspension-feeders are poorly known (Ribes et al. 2000). Previous studies have estimated annual carbon requirements for different species of suspension-feeders by integrating information on ingestion, absorption, faeces production, respiration and secondary production into a carbon or energy budget. Some of these species include types of: bivalves (Riisgard &

Randlov 1981; Loo & Rosenberg 1996), polychaetes (Newell et al. 1982), corals (Fabricius et al. 1998; Ribes et al. 2003) and other cnidarians (Coma et al. 1998). Also, several studies have estimated the energy costs of the various physiological activities in sponges (e.g. Stuart & Klumpp 1984; Barthel 1986; Ribes et al. 1999), but only a few have integrated calculations for energy demand into a more complete energy model (Reiswig 1981; Barthel 1988; Koopmans et al. 2010). Such models or budgets can examine how matter and energy are invested in different processes such as pumping, reproduction, growth and maintenance of sponges (Coma et al. 1998; Koopmans et al. 2010). In the remainder of this chapter, I will integrate all the information gathered on the feeding ecology of the three species of demosponges (*Crella incrustans*, *Haliclona venustina* and *Strongylacidon* sp.) that were examined over a two-year period (Chapter 6), and incorporate it into a preliminary carbon budget (indicative of an energy budget) for each species. These budgets will then be used to deduce the capacity of carbon obtained via heterotrophic suspension-feeding to support sponge metabolism, as well as infer the potential for this carbon to support other processes such as sponge growth and reproduction.

A simplified carbon budget was constructed for each of the study species by including both direct measurements and a number of assumptions based on the general energy budget equation proposed by Crisp (1971):

$$C = P + R + G + U + F \tag{7.1}$$

Where C is consumption, P is production (growth), R is respiration, G is gonad (reproduction), U is excretion, and F is egestion. The components included in the present budget are: consumption (C) and respiration (R) which were obtained from direct measurements. Data on growth (P), reproduction (G), F (faeces) and excretion (U) were not available for the study species and thus were not included in the calculations. Given that the use of carbon/energy for basal metabolism is considered the priority in any budget, any carbon remaining can then be assumed to be available for one or more of the other processes (Muscatine et al. 1981; Muscatine et al. 1984). For the budget calculations, an annually averaged value of retention efficiency

was used per species, and all the measurements were normalised to g DW per sponge species and expressed as annual rates of carbon consumption.

7.2.1 Carbon consumption in respiration

Because only small sponge samples (< 1 cm^3) could be removed from the marine reserve, three sponge specimens of each of the study species of similar size to those surveyed, were collected from a nearby site to carry out respiration measurements in the laboratory (see Appendix B for methodological details). The specimens collected from outside the marine reserve were selected based on previous size measurements taken from *in situ* photographs of the study species (Chapter 6). The respiration rate of the whole sponge was divided by its dry weight and then converted into carbon units, using a conversion factor of 0.3182 mg C mg^{-1} O_2 (Reiswig 1981). Respiration was calculated as an annual rate of oxygen consumption for each of the study species assuming that it remains constant over the year. Here it is important to mention that this assumption is probably inaccurate since respiration is likely to change over different times of year and in response to changes in temperature and feeding activity (Riisgård & Larsen 2000; Coma et al. 2002). However, there were no practical alternatives to this approach.

7.2.2 Carbon acquisition by feeding

Consumption was defined as the amount of carbon gained by the removal of picoplankton from seawater. It refers to the amount of food that is available in the surrounding water, which then enters the inhalant canal of the sponge and is finally taken into its cells. The amount of carbon consumed was calculated for each type of picoplankton by multiplying the number of cells retained (Equation 6.1) by the mean quantity of carbon contained in each cell according to its type (using carbon conversions from the literature), as described in Chapter 4. The amount of carbon gained by each species was normalised to grams of dry tissue weight of sponge, and expressed as the annual amount of carbon gained by each species (mg C g DW y^{-1}). The same sponge specimens collected for the respiration experiments were subsequently used for determinations of weight and volume (Appendix C).

7.2.3 Carbon requirements of the study species

The average annual consumption of carbon by *C. incrustans* from the three types of picoplankton it filtered was 53.9 mg C g DW $yr^{-1}$and the annual respiration rate was 752.6 mg C g DW yr^{-1}. Considering the estimated respiration rate of the sponge per year, and the annual retention efficiency (53%), it means that feeding on picoplankton had the potential to supply a very small amount (3.1%) of the sponge's annual respiratory carbon demands.

The budget for *H. venustina* was somewhat different from that of *C. incrustans*. This species had a much higher annual carbon consumption from the picoplankton it filtered with a value of 175.4 mg C g DW yr^{-1}, and the respiration rate for this species was 669 mg C g DW yr^{-1}. Considering the estimated respiration rate of the sponge per year and an annual retention efficiency of 45%, feeding on picoplankton contributed to 11.8% of the sponge's annual respiratory carbon demand.

The budget of *Strongylacidon* sp. showed that this species had an annual carbon consumption of 47.2 mg C g DW $yr^{-1}$from the three types of picoplankton it filtered, and the respiration rate of this species was 529.6 mg C g DW yr^{-1}. Considering the estimated respiration rate of the sponge per year and an annual retention efficiency of 37%, feeding on picoplankton therefore contributed only a very small proportion (3.3%) of the respiratory carbon demand.

7.2.4 The importance of feeding on picoplankton to the metabolism of temperate sponges

It is not surprising that the proposed budgets vary between species since the results from Chapter 6 previously showed that the feeding activity of the three species varied significantly throughout the year, and the retention of food particles was different for each species and it varied among sampling periods. The values presented above on the contribution of the respiratory demand of the three species imply that respiration is not supported fully by feeding on picoplankton. This means that these particles cannot be the only source of food in the diet of the study species. Therefore, the study species must be obtaining carbon from elsewhere in order to support their remaining

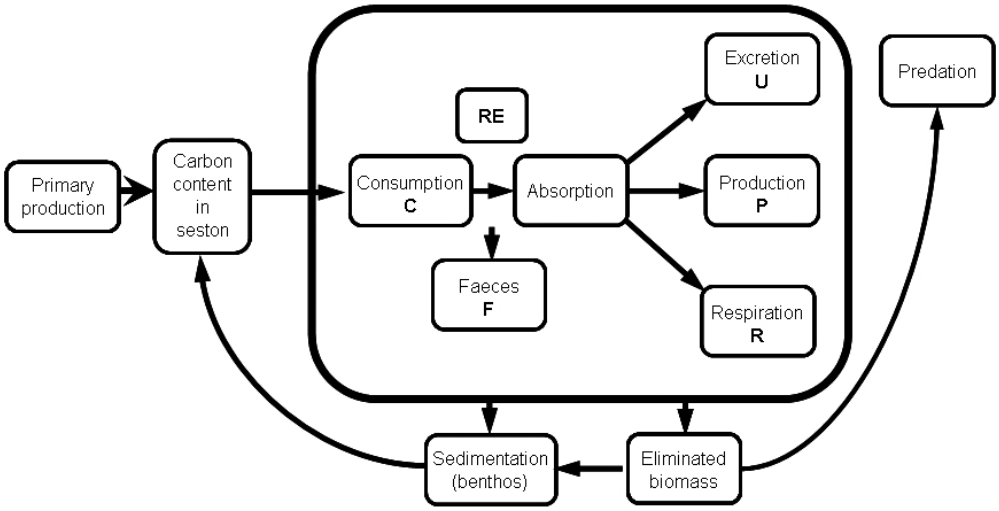
metabolic requirements, as well as growth and reproduction. Other possible sources of carbon include dissolved organic carbon (DOC) and other forms of particulate organic carbon (POC), both live (i.e. planktonic organisms) and detrital (Ribes et al. 2000). The ability of sponges to efficiently capture other types of plankton (< 10 µm) such as pico- and nanoeukaryotes has been documented (Pile 1997, 2005; Caralt et al. 2008), and some authors have been able to distinguish different populations of viruses in natural seawater samples and their removal by sponges (Marie et al. 1999; Patten et al. 2006). Heterotrophy is a common form of carbon acquisition in sponges, either via consumption of microbes from seawater or via microbial uptake of DOC (Yahel et al. 2003).

Crucially, it has been suggested that only sponges with large amounts of sponge-associated bacteria can utilise dissolved organic matter (DOM), but that in these cases this 'food source' supplies the majority of the carbon and energy needs of the sponge (e.g. Yahel et al. 2003). For this reason, the utilisation of DOM by sponges should be more appropriately described as 'DOM-feeding by the sponge–microbe association' (de Goeij et al. 2008a; 2008b). It should also be considered that the sponges' nutritional needs may be supplemented by their microbial symbionts, and in particular photosynthetically-active microbes (Barthel 1988). Indeed in some sponges, carbon nutrition depends heavily on cyanobacterial symbionts that enable the sponge to obtain the major portion of its carbon photosynthetically; this is referred to as phototrophy (Wilkinson 1983; Cheshire & Wilkinson 1991). Some temperate sponges are known to contain photosynthetic symbionts (Roberts et al. 1999; Taylor et al. 2004), and one temperate species has been demonstrated to sustain itself photosynthetically through association with symbionts (Cheshire et al. 1995). The presence of symbionts in the sponges was not assessed in this work and therefore the potential contribution of photosynthetic carbon cannot be determined here. However, the presence and function of both phototrophic and heterotrophic microbes in the sponges studied here are important topics for future research.

7.2.5 The ecological role of sponges in carbon and nutrient flow

Here I describe each of the components presented in the carbon-flow diagram (Fig. 7.1) in terms of the available information on the energetics or carbon demands of sponges. Primary production in the oceans is the first step in the food chain and results from allochthonous nutrient inputs to the euphotic zone (new production) and from nutrient recycling in the surface waters (regenerated production) (Eppley & Peterson 1979). The seston includes free-living bacteria, pico- and nanoeukaryotes, dinoflagellates, diatoms, ciliates and POC (suspended detrital particles with their associated bacteria, faecal material from micro and mesozooplankton, dead phytoplankton and bacterial cells) (Heymans & Baird 1995). In sponges, the retained food is then used for two purposes: to supply nutrients for sponge biomass and to supply energy, in which case the carbon is converted to carbon dioxide, which is assumed to take place via aerobic metabolism (Koopmans et al. 2010). Of the particles that sponges remove, a certain portion will be taken up by the sponge (absorbed), and another will leave the sponge again as either faeces or pseudo-faeces, which consist of undigested material exocytosed as larger membrane-covered units (Barthel 1988). The absorption component represents the carbon gained from the ingested food that will be destined for physiological processes, with the most important being respiration, production (growth and reproduction), and excretion.

Oxygen consumption is a measure of respiration rate that can be converted into carbon or energy equivalents, and represents a measure of that part of the food intake that is required to provide energy to support life processes (Clarke 1991). Oxygen consumption, calculated on the basis of respiration rate, has been measured in different sponge species (e.g. Reiswig 1974; Wilkinson 1983; Kowalke 2000; Hadas et al. 2008), but only a few studies having examined the respiration rates of sponges in temperate ecosystems (e.g. Barthel 1988; Thomassen & Riisgard 1995; Coma et al. 2002; this study; Murray 2009). Respiration is the component that may cause significant modifications to the budget, as the values are typically obtained from laboratory measurements (as they were here) where the sponges may be stressed and are not necessarily exposed to natural variations such as



	C	RE	F	A	P	R	U
Crella incrustans	54	53	UNK	UNK	UNK	753	UNK
Haliclona venustina	175	45	UNK	UNK	UNK	669	UNK
***Strongylacidon* sp.**	47	37	UNK	UNK	UNK	530	UNK

Figure 7.1: Carbon flow diagram modified from Loo & Rosenberg (1996), integrating the measured and the estimated components of the three study species into a preliminary carbon budget. The values of consumption and respiration are expressed in mg C g DW yr^{-1}, DW. UNK=unknown

seasonal food supply and water temperate; such factors can alter respiration rates considerably (Coma et al. 1998). After respiration the most likely uses of carbon are for growth and reproduction. While growth was not measured during this study, there are various studies that have recorded the growth rates of sponges in the field (e.g. Ayling 1983; Barthel 1986;Duckworth 2003; Duckworth et al. 2004; Knott et al. 2006). Furthermore, growth varies with time of year, with lower growth rates generally being measured in winter (De Caralt et al. 2008; Koopmans & Wijffels 2008). In terms of carbon use, growth determines the percentage increase in body mass over time, and the temporal variation in growth of some species may result from seasonal differences in food availability (Duckworth & Battershill 2001). Reproduction of sponges has been studied in a moderate number of species world-wide compared with other taxa (Ilan et al. 2004). In most sponges maturation events usually take place seasonally (Reiswig 1981). In a study of two cold water sponges, Barthel (1986) suggested that adult sponges degenerate after reproduction in summer. A Mediterranean sponge, during its reproductive period (spring–summer), experiences the reduction of the number of functional pumping units and the transformation of choanocyte chambers into spermatogonia (de Caralt et al. 2007). Studies looking at reproduction in New Zealand sponges indicated that some species seem to be reproductively active in summer and early autumn correlating with periods of relatively poor growth, and other species appear to be reproductively active throughout the year (Ayling 1980; Duckworth & Battershill 2001).

When nutrients in the water column are depleted, degeneration and disintegration of sponges provide a nutrient pulse to primary producers (Barthel 1988). Sponges remove part of the regenerated primary production during the time of their strongest growth and release nutrients into the euphotic zone, thus stabilising the regenerative cycle and reducing sedimentation of phytoplankton to the benthos (Barthel 1986). Sedimentation accounts for losses of organic matter, where unutilised production is assumed to become incorporated into sedimentary POC (Heymans & Baird 1995). Sponges enhance sedimentation with the particles that pass through them undigested, and this process influences the flow of energy and matter from the water column to the benthos (Barthel 1988). The eliminated biomass accounts for

the sponge biomass that will enter the sedimentation process and the detrital food chain, as well as the amount of biomass that will be reconverted into dissolved plant nutrients and DOM (Barthel 1988), and the biomass lost by predation. Previous field studies carried out in New Zealand have suggested that only a minor share of sponge biomass is transported directly to higher trophic levels (Ayling 1981; Duckworth & Battershill 2001). The majority seems to be recycled within the euphotic zone, or to enter the detrital food chain (Barthel 1988).

7.3 Future research and concluding remarks

The budgets proposed here must be considered as preliminary and approximate due to the number of assumptions and limitations involved. To be more comprehensive, such budgets should include total consumption, and must include the capture of both dissolved and particulate resources (Reiswig 1981). One of the main conclusions from the carbon budget calculations was that feeding on picoplankton had the potential to supply only a very small amount of the sponge's annual respiratory carbon demands, meaning that the diet of these species was not sufficiently resolved, therefore leaving a deficit in the budget that can only be satisfied by other sources. Possible sources of this carbon include DOC and photosynthethic symbionts. However, it is also important to note that the study species might have been stressed when the respiration rates were measured in the laboratory, so resulting in artificially high respiration values. Given that respiration is such an important aspect of the carbon budget, future work should examine the seasonal variation in the respiration rate of the study species using an *in situ* technique to better represent the physiological energetics of sponges in their natural habitat, and minimise the impacts of handling-related stress (Coma et al. 2002).

Although the data included in the budgets here were measured as precisely as possible, some parameters remain unknown, such as the influence of water flow on picoplankton capture and respiration rates. Also, other measurements such as estimates of growth and reproduction are needed in order to build a more comprehensive budget. A non-destructive method for measuring growth *in situ* that could be applied in the study area would be to take

monthly photographs to monitor volume and sponge surface area changes in time (Garrabou & Zabala 2001; Koopmans & Wijffels 2008). There is also the need to measure carbon content in sponge tissues by using an element analyser (Koopmans et al. 2010) or by stable isotope analysis (Topçu et al. 2010). The resulting values can be used to obtain more insight in the carbon usage of sponges in relation to energy and biomass increase; similar approaches can also be applied to our understanding of nitrogen acquisition and usage.

The preliminary budgets presented here provide an important first step towards understanding the nutritional requirements of sponges. As mentioned previously, picoplanktonic organisms are one of the major components of food webs and play key roles in biogeochemical cycles and energy flow. The results from this thesis showed that sponge assemblages on the subtidal rocky reef studied are capable of consuming a considerable proportion of the available picoplankton, and are therefore responsible for the transfer of large amounts of carbon from the water column to the benthos. However, while the study species consume a great amount of picoplankton this is still inadequate for supporting their basal metabolism, since feeding on picoplankton had the potential to supply only a small amount of the sponge's annual respiratory carbon demands. This also brings the idea of niche partitioning and the question of whether or not there was any differential utilisation of picoplankton by the three species used in the carbon budgets. Different retention efficiencies were observed among the three study species for the three types of picoplankton, which is in agreement with the results obtained from Chapter 3. Considering the annual retention efficiency for each type of picoplankton by the study species, *Strongylacidon* sp. may fit within group I where species had higher retention rates of *Prochlorococcus* and *Synechococcus*, and lower retention of heterotrophic bacteria. *C. incrustans* and *H. venustina* may fit within group II where species had higher retention of *Prochlorococcus* and heterotrophic bacteria, and removed less *Synechococcus*. However, a further analysis is needed to integrate these three species with the species from Chapter 3 in order to support the hypothesis of partitioning of food resources between these co-existing sponge species.

The ability of sponges to directly consume picoplankton represents a cru-

cial step in the understanding of energy flow and ecosystem functioning. I have mentioned that sponges play an important role in the flow of carbon through marine ecosystems as they have the ability to control the cycling of nutrients, organic matter, plankton and detritus. Hence, another important aspect studied in this thesis was nutrient availability. The results from this thesis showed that there were seasonal changes in nutrient and food availability, but it remains to be elucidated if the study species show seasonal trends in respiration rate, and if an increase/decrease in these parameters is related to an increase/decrease in water temperature, nutrient levels and suspended particulate matter. The broader implications of the results obtained from the nutrient uptake/release of the study species could be important, since nutrient fluxes relate to the ecological importance of sponges, specially due to their release of ammonium and nitrate into the water column as these nutrients are considered to be the most important sources of regenerated and newly produced nitrogen for biological processes. Indeed, the information gathered throughout the duration of this thesis can be used to inform future studies of the feeding ecology and physiology of these important benthic organisms, and to recognise the important role that sponges play in the balance and dynamics of carbon and nutrients in the water column.

7.4 References

— Ayling A.L. (1980). Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine Demospongiae. Biological Bulletin, 158, 271-282.

— Ayling A.L. (1981). The role of biological disturbance in temperate subtidal encrusting communities. Ecology, 62, 830-847.

— Ayling A.L. (1983). Factors affecting the spatial distributions of thinly encrusting sponges from temperate waters. Oecologia, 60.

— Barthel D. (1986). On the ecophysiology of the sponge *Halichondria panicea* in Kiel Bight .1. Substrate-specificity, growth and reproduction. Marine Ecology-Progress Series, 32, 291-298.

— Barthel D. (1988). On the ecophysiology of the sponge *Halichondria panicea* in Kiel Bight .2. Biomass, production, energy budget and integration in environmental processes. Marine Ecology-Progress Series, 43, 87-93.

— Caralt S.D., Uriz M.J. & Wijffels R.H. (2008). Grazing, differential size-class dynamics and survival of the Mediterranean sponge *Coticium candelabrum*. Marine Ecology and Progress Series, 360, 97-106.

— Cheshire A.C., Butler A.J., Westphalen G., Rowland B., Stevenson J. & Wilkinson C.R. (1995). Preliminary study of the distribution and photophysiology of the temperate phototrophic sponge *Cymbastela* sp from South Australia. Marine and Freshwater Research, 46, 1211-1216.

— Cheshire A.C. & Wilkinson C.R. (1991). Modelling the photosynthetis production by sponges on Davies Reef, Great Barrier Reef. Marine Biology, 109, 13-18.

— Clarke A. (1991). What is cold adaptation and how should we measure it. American Zoologist, 31, 81-92.

— Coma R., Ribes M., Gili J.M. & Zabala M. (1998). An energetic approach to the study of life-history traits of two modular colonial benthic invertebrates. Marine Ecology-Progress Series, 162, 89-103.

— Coma R., Ribes M., Gili J.M. & Zabala M. (2002). Seasonality of *in situ* respiration rate in three temperate benthic suspension feeders. Limnology and Oceanography, 47, 324-331.

— Crisp D.J. (1971). Energy flow measurements. In: Methods for the study of marine benthos. IBP Handbook No. 16 (eds. Holme NA & McIntyre AD). Blackwell Oxford, pp. 197-279.

— De Caralt S., Uriz M.J. & Wifffels R.H. (2008). Grazing, differential size-class dynamics and survival of the Mediterranean sponge *Corticium candelabrum*. Marine Ecology-Progress Series, 360, 97-106.

— de Caralt S., Uriz M.J. & Wijffels R.H. (2007). Vertical transmission and successive location of symbiotic bacteria during embryo development and larva formation in *Corticium candelabrum* (Porifera : Demospongiae). Journal of the Marine Biological Association of the United Kingdom, 87, 1693-1699.

— de Goeij J.M., Moodley L., Houtekamer M., Carballeira N.M. & van Duyl F.C. (2008a). Tracing C-13-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM feeding. Limnology and Oceanography, 53, 1376-1386.

— de Goeij J.M., van den Berg H., van Oostveen M.M., Epping E.H.G. & Van Duyl F.C. (2008b). Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. Marine Ecology-Progress Series, 357, 139-151.

— Duckworth A.R. (2003). Effect of wound size on the growth and regeneration of two temperate subtidal sponges. Journal of Experimental Marine Biology and Ecology, 287, 139-153.

— Duckworth A.R. & Battershill C.N. (2001). Population dynamics and chemical ecology of New Zealand demospongiae *Latrunculia* sp nov and *Polymastia croceus* (Poecilosclerida : Latrunculiidae : Polymastiidae). New Zealand Journal of Marine and Freshwater Research, 35, 935-949.

— Duckworth A.R., Battershill C.N. & Schiel D.R. (2004). Effects of depth and water flow on growth, survival and bioactivity of two temperate sponges cultured in different seasons. Aquaculture, 242, 237-250.

— Eppley R.W. & Peterson B.J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. Nature, 282, 677-680.

— Fabricius K., Yahel G. & Genin A. (1998). *In situ* depletion of phytoplankton by an azooxanthellate soft coral. Limnology and Oceanography, 43, 354-356.

— Garrabou J. & Zabala M. (2001). Growth dynamics in four Mediterranean demosponges. Estuarine Coastal and Shelf Science, 52, 293-303.

— Hadas E., Ilan M. & Shpigel M. (2008). Oxygen consumption by a coral reef sponge. Journal of Experimental Biology, 211, 2185-2190.

— Heldal M., Scanlan D.J., Norland S., Thingstad F. & Mann N.H. (2003). Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. Limnology and Oceanography, 48, 1732-1743.

— Heymans J.J. & Baird D. (1995). Energy-flow in the Kromme Estuarine ecosystem, St Francis Bay, South Africa. Estuarine Coastal and Shelf Science, 41, 39-59.

— Ilan M., Gugel J. & Van Soest R.W.M. (2004). Taxonomy, reproduction and ecology of new and known Red Sea sponges. Sarsia, 89, 388-410.

— Knott N.A., Underwood A.J., Chapman M.G. & Glasby T.M. (2006). Growth of the encrusting sponge *Tedania anhelans* (Lieberkuhn) on vertical and on horizontal surfaces of temperate subtidal reefs. Marine and Freshwater Research, 57, 95-104.

— Koopmans M., Martens D. & Wijffels R.H. (2010). Growth Efficiency and Carbon Balance for the Sponge *Haliclona oculata*. Marine Biotechnology, 12, 340-349.

— Koopmans M. & Wijffels R.H. (2008). Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida). Marine Biotechnology, 10, 502-510.

— Kowalke J. (2000). Ecology and energetics of two Antarctic sponges. Journal of Experimental Marine Biology and Ecology, 247, 85-97.

— Loo L.O. & Rosenberg R. (1996). Production and energy budget in marine suspension feeding populations: *Mytilus edulis*, *Cerastoderma edule*, *Mya arenaria* and *Amphiura filiformis*. Journal of Sea Research, 35, 199-207.

— Marie D., Brussaard C.P.D., Thyrhaug R., Bratbak G. & Vaulot D. (1999). Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied Environmental Microbiology, 65, 45-52.

— Murray H.J. (2009). Oxygen consumption rates of sponges and the effect of UV-B radiation and sediment. Masters Thesis, Victoria University of Wellington, Wellington. 150 p.

— Muscatine L., Falkowski P.G., Porter J.W. & Dubinsky Z. (1984). Fate of Photosynthetic fixed carbon in light-adapted and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proceedings of the Royal Society of London Series B-Biological Sciences, 222, 181-202.

— Muscatine L., McCloskey L.R. & Marian R.E. (1981). Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnology and Oceanography, 26, 601-611.

— Newell R.C., Field J.G. & Griffiths C.L. (1982). Energy balance and significance of microorganisms in a kelp bed community. Marine Ecology-Progress Series, 8, 103-113.

— Partensky F., Hess W.R. & Vaulot D. (1999). *Prochlorococcus*, a Marine Photosynthetic Prokaryote of Global Significance. Microbiology and Molecular Biology Reviews, 63, 106-127.

— Patten N.L., Seymour J.R. & Mitchell A.G. (2006). Flow cytometric analysis of virus-like particles and heterotrophic bacteria within coral-associated reef water. Journal of the Marine Biological Association of the United Kingdom, 86, 563-566.

— Pile A.J. (1997). Finding Reiswig's missing carbon: quantification of sponge feeding using dual-beam flow cytometry. In: Proceedings of the 8th International Coral Reef Symposium, pp. 1403-1410.

— Pile A.J. (2005). Overlap in diet between co-occurring active suspension feeders on tropical and temperate reefs. Bulletin of Marine Science, 76, 743-749.

— Reiswig H.M. (1974). Water transport respiration and energetics of 3 tropical marine sponges. Journal of Experimental Marine Biology and Ecology, 14, 231-249.

— Reiswig H.M. (1981). Partial carbon and energy budgets of the bacterio sponge *Verongia fistularis* Porifera Demospongiae in Barbados West-Indies. Marine Ecology, 2, 273-294.

— Ribes M., Coma R. & Gili J.M. (1999). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology-Progress Series, 176, 179-190.

— Ribes M., Coma R., Gili J.M., Svoboda A., Julia A. & Parera J. (2000). A 'semi-closed' recirculating system for the *in situ* study of feeding and respiration of benthic suspension feeders. Scientia Marina, 64, 265-275.

— Ribes M., Coma R. & Rossi S. (2003). Natural feeding of the temperate asymbiotic octocoral-gorgonian *Leptogorgia sarmentosa* (Cnidaria : Octocorallia). Marine Ecology-Progress Series, 254, 141-150.

— Riisgård H.U. & Larsen P.S. (2000). Comparative ecophysiology of active zoobenthic filter feeding, essence of current knowledge. Journal of Sea Research, 44, 169-193.

— Riisgard H.U. & Randlov A. (1981). Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentrations. Marine Biology, 61, 227-234.

— Roberts D.E., Cummins S.P., Davis A.R. & Pangway C. (1999). Evidence for symbiotic algae in sponges from temperate coastal reefs in New South Wales, Australia. Memoirs of the Queensland Museum, 44, 493-497.

— Stuart V. & Klumpp D.W. (1984). Evidence for food-resource partitioning by kelp-bed filter feeders. Marine Ecology Progress Series, 16, 27-37.

— Taylor M.W., Schupp P.J., Dahllof I., Kjelleberg S. & Steinberg P.D. (2004). Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. Environmental Microbiology, 6, 121-130.

— Thomassen S. & Riisgard H.U. (1995). Growth and energetics of the sponge *Halichondria panicea*. Marine Ecology-Progress Series, 128, 239-246.

— Topçu N.E., Perez T., Gregori G. & Harmelin-Vivien M. (2010). *In situ* investigation of *Spongia officinalis* (Demospongiae) particle feeding: Coupling flow cytometry and stable isotope analysis. Journal of Experimental Marine Biology and Ecology, 389, 61-69.

— Wilkinson C.R. (1983). Net primary productivity in coral reef sponges. Science, 219, 410-412.

— Yahel G., Sharp J.H., Marie D., Hase C. & Genin A. (2003). *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnology and Oceanography, 48, 141-149.

Appendix A

Study species photographs

(a) *Dysidea* sp.

(b) *Leucosolenia echinata*

(c) *Leucetta* sp.

(d) *Haliclona* sp.

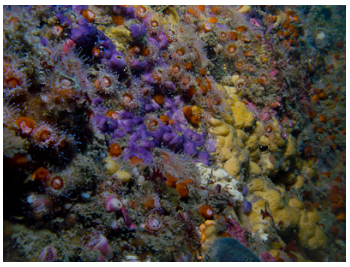
(e) *Polymastia* sp.

(f) *Tethya bergquistae*

(g) *Crella incrustans*

(h) *Haliclona venustina*

(i) *Strongylacidon* sp.



(j) *Plakina* sp.

Figure A.1: Sponge species photos

Appendix B

Respiration Protocol

Three sponge specimens of each of the study species (*Crella incrustans*, *Haliclona* v*enustina* and *Strongylacidon* sp.) were collected to carry out respiration measurements in the laboratory. The laboratory work was conducted at the Victoria University Coastal Ecology Lab (VUCEL) in Wellington, New Zealand. After collection, sponges were carefully transported to the laboratory and were placed in a tank with a constant flow of unfiltered seawater (pumped directly from the sea), and left to acclimatise for three days. To ensure that all the specimens were in good condition before the respiration experiments were performed, a small amount of fluourescein dye was released next to each specimen to corroborate pumping activity.

The experiments were conducted in winter when water temperature was 12.4 ± 0.8 °C (Berman unpub. data). The experimental set up consisted of a glass aquarium tank (water bath) half-filled with seawater, three acrylic respiration chambers, a magnetic stirrer, oxygen electrodes, a lamp, a clamp and a computer. Prior to the beginning of the experiment, the respiration chambers were cleaned and the internal volume was calculated. Then, one at a time, sponges were placed inside an open respiration chamber and maintained in individual buckets with a constant supply of flow-through filtered seawater (filtered by a 1 µm mesh size filter) to clean off debris, microorganisms and sediment. All experiments were conducted inside a cold room where the ambient temperature could be controlled and adjusted to the water temperature coming in from the seawater supply.

To measure oxygen consumption, a single sponge specimen was placed in a respiration chamber that was sealed making sure no air bubbles were trapped in the chamber with the sponge. Then, the chamber with the sponge inside was mounted on top of a magnetic stirrer to guarantee mixing of the water, while also keeping the sponge active. The chamber was fitted with oxygen electrodes that were used to monitor changes in oxygen concentration. The oxygen electrodes were held in a clamp during all the respiration experiments because they are very delicate and easy to break, hence care was taken when handling them.

Both light (with the lamp switched on) and dark (chamber wrapped in foil paper) experiments were conducted for each sponge specimen. This was done to determine if there was a rate of oxygen production (net photosynthesis), considering the possibility of associated microbes in the study species. Two readings (one light, one dark) of each sponge specimen were taken for long enough for the oxygen levels in the chamber to drop to about 80% (usually for $20-35$ min). The data collected were transferred to an excel data sheet. The rate of oxygen depletion in the chambers was plotted against time to check if it was a linear decrease. Dissolved oxygen was measured with an IBOX 3-fiber-optic oxygen meter (PreSens GmbH, Germany) respirometer oxygen meter. The rate of oxygen per hour used in the chamber was calculated, and this was correlated back to the volume of water in the chamber so the oxygen per ml water could be calculated, and then related to how much oxygen was used per gram of sponge tissue. After all the experimental runs, sponge specimens were used for determinations of weight and volume.

Appendix C

Sponge volume and mass protocol

After the respiration experiments, the sponge specimens were subsequently used for determinations of weight and volume. Volume measurements were carried out by displacement volume where sponge specimens were tied to thin thread and suspended in a known mass or volume of seawater. The difference in mass was multiplied by the density of the water resulting in the sponge volume.

For weight determinations, clean aluminium trays were labelled and weighed. Each sponge specimen was dabbed with absorbent tissue to remove excess water and weighed in the tray to obtain the wet weight (WW). Then, samples were placed in a drying oven at 60 ºC for 48 hours to remove all moisture. After this time samples were weighed again to obtain the dry weight (DW). Next, samples were placed in clean and labelled metal crucibles and transferred to the furnace where they were heated at 480 ºC for 6 hours. After this time the samples were weighed again to obtain the ash-free weight (AFDW).

Appendix D

Papers published and submitted

Below I indicate the current and intended publication status of my thesis chapters:

Published

Chapter 2 Perea-Blázquez A., Price K., Davy S.K. & Bell J.J. (2010). Diet Composition of two temperate calcareous sponges: *Leucosolenia echinata* and *Leucetta* sp. from the Wellington South Coast, New Zealand. The Open Marine Biology Journal, 4, 65-75 (see below).

Chapter 5 Perea-Blázquez A., Davy S.K. & Bell J.J. Nutrient utilisation by shallow water temperate sponges in New Zealand. Hydrobiologia, 10.1007/s10750-011-0798-x, 1-14 (see below).

In review

Chapter 3 Perea-Blázquez A., Davy S.K. & Bell J.J. Differential food resource use by temperate subtidal sponges. In review, submitted to: Journal of Experimental Marine Biology and Ecology, 05/07/2011

Chapter 4 Perea-Blázquez A., Davy S.K. & Bell J.J. Estimates of Particulate Organic Carbon Flowing from the Pelagic Environment to the Benthos through Sponge Assemblages. In review, submitted to: PLoS ONE, 15/07/2011

To be submitted

Chapter 6 Perea-Blázquez A., Davy S.K. & Bell J.J. Fluctuations in food utilisation by three common species of demosponges over time.

Chapter 7 Perea-Blázquez A., Davy S.K. & Bell J.J. The importance of picoplanktonic feeding to temperate sponges, and their ecological significance.

Diet Composition of Two Temperate Calcareous Sponges: *Leucosolenia echinata* and *Leucetta* sp. from the Wellington South Coast, New Zealand

[1]*Centre for Marine Environmental and Economic Research, School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand*

[2]*Flow Cytometry Suite, Malaghan Institute of Medical Research, Wellington, New Zealand*

Abstract:

Leucosolenia echinata *Leucetta*

Prochlorococcus *Synechococcus*

Prochlorococcus *Synechococcus*

Keywords:

INTRODUCTION

et

al

Pericharax heteroraphis
in situ

µ

Prochlorococcus µ

a *b*
Synechococcus µ

µ

1874-4508/10

Hydrobiologia
DOI 10.1007/s10750-011-0798-x

SPONGE RESEARCH DEVELOPMENTS

Nutrient utilisation by shallow water temperate sponges in New Zealand

Alejandra Perea-Blázquez · Simon K. Davy · James J. Bell

Received: 24 February 2011 / Accepted: 13 June 2011

Abstract Major nutrients such as phosphate, nitrate, ammonium and silicate, are involved in the metabolic processes of marine organisms. Sponges take up and produce inorganic nutrients and the extent at which they affect the budgets available for other organisms has received little attention. For this reason, we investigated nutrient fluxes for several sponge species in order to estimate whether sponges were net producers or consumers of nutrients from the water column, and how these patterns changed over time. Nutrient fluxes were examined on the south coast of Wellington, New Zealand. For the nutrient analysis (nitrate, nitrite, ammonium, phosphate and silicate), water samples were collected in situ from the inhalant and exhalant water of different sponge species. Samples were analysed both in a multi-species survey and over a two-year period for three other species to determine any temporal changes in fluxes. Our results yielded significant differences in nutrient concentrations between the inhalant and exhalant water for some of the species, but there was no clear pattern associated with the time of year. The levels of dissolved inorganic nutrients in the ambient water varied considerably over the 2-year study period. It is possible that a lack of a clear pattern of nutrient uptake/release of nutrients in some of the study species, and the fact that not all species showed significant uptake/release at different times of the year, may be related to high levels of temporal and spatial variation in the ambient nutrient availability, as well as other temporal fluctuations in parameters, such as water temperature, sponge size, and concentration of food in the water column. Finally, we believe that the activity of specific microbial communities associated with these sponges may be important in explaining the fluxes we have reported.

Keywords Nutrient fluxes · In situ · Sponges · Temporal · Variation · Temperate ecosystem

Guest editors: M. Maldonado, X. Turon, M. A. Becerro & M. J. Uriz / Ancient animals, new challenges: developments in sponge research

A. Perea-Blázquez (✉) · S. K. Davy · J. J. Bell
Coastal Ecology Laboratory, School of Biological Sciences, Victoria University of Wellington, 396 The Esplanade, Island Bay, Wellington 6023, New Zealand
e-mail: alejandra.perea@vuw.ac.nz

S. K. Davy · J. J. Bell
Victoria University of Wellington, School of Biological Sciences, New Kirk Building, Kelburn Pde, Kelburn Campus, PO Box 600, Wellington, New Zealand

Introduction

Marine organisms utilise major nutrients including phosphate, nitrate, ammonium and silicate for metabolic functions. Nitrogen has been intensively studied due to its importance in nutrient fluxes and transformations that occur in the marine environment

Published online: 07 July 2011