Coral-bacteria Associations: Determining the Role of Chemotaxis

Honours Thesis 2010

Jessica Tout
10418313

Plant Functional Biology and Climate Change Cluster, Department of Environmental Science, University of Technology, Sydney, Australia.

Supervisors: Dr Justin Seymour
Professor Peter Ralph

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**Declaration**

This thesis contains no material that has been accepted for the award of any other degree or diploma, and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text.

Jessica Tout
10418313

**New format of thesis**

This thesis follows the instructions suggested by Professor Peter Ralph and Dr Justin Seymour and agreed to by UTS DES Honours committee, accepted via email on 30/07/2010.

This document contains a general background to the thesis, a manuscript with an abstract, introduction, results, discussion and conclusion in a form ready for submission to Marine Ecology Progress Series journal along with an appendix containing details of method development and pilot studies.
**Table of Contents**

Table of contents ........................................................................................................... 3  
Manuscript ....................................................................................................................... 4  
Acknowledgements ......................................................................................................... 4  
  General background to thesis....................................................................................... 5  
  Abstract......................................................................................................................... 8  
  Introduction .................................................................................................................. 10  
  Materials and Methods ............................................................................................... 13  
  Results ......................................................................................................................... 19  
  Discussion ..................................................................................................................... 29  
  Conclusion .................................................................................................................... 39  
  References .................................................................................................................... 41  

Appendix to thesis .......................................................................................................... i  
  Method development and Pilot studies ........................................................................ i  
    Methodological details, not provided in the manuscript............................................. xii  
    Additional Studies ................................................................................................... xv  

CD .................................................................................................................................. xiv  
  Flow Cytometry enumeration ..................................................................................... 
  Statistical Analysis ..................................................................................................... 

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**General background to thesis**

The ‘grandfather of microbiology’ and the inventor of the microscope Antony van Leeuwenhoek was the first to observe and describe bacterial movement in 1683. He described what he saw in the dental plaque of an old man as “animalcules”, which were in fact bacteria (Lux and Shi 2004). By 1883 it was found that bacterial movement was not random and arbitrary, but directed towards nutrient cues, and this behaviour was termed ‘chemotaxis’ (Engelmann 1883; Pfeffer 1884; Lux & Shi 2004; Englert et al. 2009). Pfeffer was the first to illustrate the capillary assay, which led to remarkable discoveries about chemotaxis during the next century (Englert et al. 2009).

Almost one hundred years later, Julius Adler described the behaviour of *Escherichia coli* by showing that bacteria use specific receptors known as ‘chemoreceptors’ to recognise and swim towards chemicals (Adler 1966; Adler 1969). Adler (1973) standardised and popularised Pfeffer’s capillary assay, which identified compounds such as amino acids and sugars as ‘chemoattractants’ (Englert et al. 2009). Over the past three decades, the examinations of the chemoattractants driving chemotactic responses in several groups of, primarily enteric bacteria have provided fundamental knowledge about the ecology and biochemistry of bacteria (Bell & Mitchell 1972; Mesibov & Adler 1972). For example, Adler and Mesibov (1972) showed that L-amino acids in proteins were linked to a range of amino acids, which acted as positive attractants or repellents for *E. coli*.

During the 1970’s, Berg and Koshland found that bacteria have a memory which enables them to compare concentrations in their surrounding environment, as they swim through it (Englert et al. 2009). Today, chemotaxis describes a behavioural response, as well as the chemical mechanism enabling bacteria to improve their adaptation to their habitats in response to chemical stimuli (Lux and Shi, 2004; Seymour et al 2008). It has been proposed that chemotaxis is the first step in bacterial ‘biocommunication’ where sign-mediated interactions occur with chemical molecules known as chemoattractants (Witzany 2010). Bacteria not only respond to chemoattractants, but they can in fact trigger a chemotactic response as they
secrete chemicals themselves, causing biocommunication and interactions with other bacteria, which is known as ‘quorum sensing’ (Witzany 2010). Bacterial clustering through quorum-sensing can lead to the development of biofilms on both biotic surfaces, such as the coral surface microlayer (CSM) (Fig. i) and abiotic surfaces like rocks (Witzany 2010).

The CSM contains mucus, which provides an excellent substrate for bacterial growth (Ducklow & Mitchell, 1979), as they can actively metabolise carbohydrates and proteins in coral mucus (Paul et al. 1986) (Fig. i). Coral mucocyte cells generate mucus which is a polysaccharide-protein (Sharon & Rosenberg, 2008) rich in sugars such as glucose, fucose, arabinose, mannose and galactose (Meikle, et al, 1988). Coral mucus represents a nutrient rich niche for bacteria growing in otherwise oligotrophic waters, such as coral reefs (Brown & Bythell 2005; Shnit-Orland & Kushmaro, 2009).

Figure i. Cross-sectional diagram depicting the structure of a scleractinian coral. This also shows the various ‘niches’ in which bacteria (in purple) can be found throughout the coral, such as the Coral Surface Microlayer (CSM), the gastrodermal cavity, and the Calcium carbonate skeleton. Zooxanthellae are represented as green dots which can be found in the gastrodermal layer (Rosenberg et al. 2007)
Since the 1980’s, standard culturing techniques were used to examine coral-associated bacteria and demonstrated that bacteria were nutritionally essential for coral metabolism (Koren & Rosenberg, 2006; Lampert et al 2008), just as coral mucus is an important nutrient for bacteria. Bacteria have a diverse array of ecological roles on coral reefs where they can be symbiotic by protecting the coral from pathogens through competition and production of antibiotic substances (Kooperman et al. 2007), and bacteria can also be pathogenic to coral, causing diseases, as well as being a food resource (Fig. ii). Through the rise of culture-independent identification, bacterial diversity has been found to be very high and demonstrates species-specificity between corals and bacteria, regardless of geographic isolation between reefs (Paul et al. 1986; Santavy, 1995; Koren & Rosenberg, 2007). However, examinations of the specific chemical attractants and chemotactic abilities of coral-associated bacteria have not yet been conducted, although there is evidence that marine bacteria can be highly chemotactic (Fig. ii) (Banin et al. 2001; Koren & Rosenberg 2006; Rosenberg et al 2007).

Figure ii. Diagram depicting the various ecological roles of marine bacteria on the Great Barrier Reef. (Webster & Hill 2007)
Coral-bacterial Associations: Determining the Role of Chemotaxis

Abstract

Bacteria play a fundamental role in determining the health and ecology of corals. The important functions of the bacterial consortia inhabiting the coral holobiont include chemical cycling, provision of a food resource for the host, as well as a potential role in coral disease. Indeed, pathogenic bacteria have been implicated as an important cause of the world-wide decline in coral health. Currently, however, little is known about the mechanisms involved in establishing an association between corals and bacteria.

The relative abundance of bacterial communities associated with the branching coral *Pocillopora damicornis* was examined under laboratory conditions in aquaria and on Heron Island, where an additional species *Montipora digitata* was also monitored. Bacterial concentrations associated with the surface of corals were compared to the surrounding water using flow cytometry. On Heron Island, there was no significant difference observed in bacterial abundances between the healthy Coral Surface Microlayer (CSM) and those of the surrounding seawater for both *P. damicornis* and *M. digitata*. However, there were significantly more bacteria associated with the CSM of diseased specimens of both *P. damicornis* and *M. Digitata*.

To examine the potential mechanism behind this coral-bacteria association, we performed chemotaxis experiments where the extent of bacterial chemotaxis towards chemicals expected to be associated with the coral holobiont were tested using a modified capillary assay. Chemoattractants tested included zooxanthellae extracellular excretions, as well as organic compounds including a variety of amino acids and sugars, dimethyl sulphide (DMS) and dimethylsulfonopropionate (DMSP). In all cases, coral associated bacteria from Heron Island exhibited significantly higher levels of chemotaxis than non-coral associated bacteria, from both the laboratory and the field. Bacterial attraction towards the sugars and amino acids tested led to bacterial concentrations that reached 176 and 186 times higher within chemoattractant chambers respectively, in comparison to background.
concentrations in the seawater. Zooxanthellae exudates invoked strong chemotactic responses, leading to bacterial concentrations within chemoattractant chambers of up to 51 times higher than background. These data indicate that coral-bacterial associations may be maintained by chemotactic responses by bacteria towards several organic compounds that are likely to be released from coral surfaces. The high levels of chemotaxis of coral-associated bacteria, relative to those found in the surrounding water column, points to a potentially important role of chemotaxis in the establishment and maintenance of coral-bacteria associations in the environment.

**Key words:** Chemotaxis, Chemoattractants, Bacterial Consortia, Coral Surface Microlayer (CSM), Holobiont
Introduction

Coral reefs are the largest living structures on Earth, with the Great Barrier Reef covering 2000 km and comprising over 3000 individual reefs formed by scleractinian corals (Rosenberg et al. 2007). Scleractinian corals are a part of the class Anthozoa, belonging to the phylum Cnideria, and produce an exoskeleton composed of calcium carbonate, which forms the foundation of coral reef ecosystems (Hutchings et al. 2008).

Scleractinian corals form a symbiotic relationship with dinoflagellate algae, from the genus Symbiodinium, which are commonly referred to as ‘zooxanthellae’. There are a variety of genetically diverse Symbiodinium clades, which inhabit scleractinian corals, and each influence the corals resilience to stress, such as its thermotolerance in different ways (Hill et al. 2009). Corals can obtain energy autotrophically when supported by the chemical products released from the zooxanthellae, which are endosymbionts within the coral’s gastrodermal cell layer (Fig. i). This symbiosis provides the coral with up to 90% of its essential nutrients, including oxygen and photosynthetically fixed carbon (Rosenberg et al. 2007) and allows both the coral and zooxanthellae to survive in otherwise oligotrophic waters (Allers et al. 2008; Houlbreque & Ferrier-pages 2009). Corals can also be heterotrophic feeders, engulfing various organisms like planktonic algae, larvae and bacteria, which pass by in the water column (Wild et al. 2004).

Zooxanthellae are not the sole symbiotic partners of coral. Coral also contain a dynamic community of heterotrophic bacteria, which colonise various niches including the mucosal layer, the gastrodermal cavity and the skeleton (Fig. i) (Paul et al. 1986; Ritchie & Smith, 1997; Rowher et al. 2001; Bourne & Munn 2005; Guppy & Bythell 2006; Koren & Rosenberg et al. 2007; Kooperman et al. 2007). The importance of microbial communities in coral physiology has only been recently acknowledged, and is now considered an integral part of the ‘coral holobiont’.

Previous research has shown that coral mucus provides an excellent substrate for bacterial growth (Ducklow & Mitchell, 1979), and that bacteria can actively
metabolise coral mucus which is also rich in zooxanthellae exudates (Disalvo, 1971; Sieburth, 1975; Ducklow, 1982; Paul et al. 1986; Brown and Bythell, 2005). Ritchie (2006) proposed that coral mucus controls the composition of the coral-associated bacteria, which can be beneficial to the coral by providing protection from pathogens through the production of antibiotics and compounds that inhibit bacterial growth, as well as supplying nutrients to the coral. It has been suggested that stress causes coral to increase mucus production within the coral surface microlayer (CSM), concurrently, the inhabitant bacteria also increase in population density and activity (Segal & Ducklow 1982; Ritchie et al. 1996a). This suggests the bacteria in the CSM are closely attuned to the holobiont’s physiological condition, as well as its metabolism (Ducklow & Mitchell 1979; Ritchie et al. 1996a). However, the ecological mechanisms supporting this association between bacteria and the CSM have not yet been fully characterised, and is the focus of our study.

The role of bacteria in the overall coral reef ecosystem remains unclear, but is suspected to be of great importance to its ecology and physiology (Rowher et al. 2002). On a large scale, heterotrophic bacteria play fundamental roles in nitrogen and carbon-cycling in coral reefs (Fig. ii) (Sorokin 1978; Webster & Hill 2007). Klaus et al (2005) proposed that coral-associated bacteria can be separated into four functional groups: 1) some bacteria can be commensal and have no affect on the coral, 2) bacteria can be a source of coral nutrition (i.e. food), 3) bacteria can be pathogenic to corals, 4) bacteria can be beneficial to corals through the ‘Coral Probiotic Hypothesis’ by promoting the growth of helpful bacteria, whilst controlling the growth of pathogenic bacteria, and adapting the bacterial consortia during times of stress to maintain coral homeostasis. The ‘Coral Probiotic hypothesis’ proposes that a coral has a diverse and dynamic microbial consortia associated with it (Reshef et al. 2006). Hence, the presence of the microbial consortia assists the coral to adapt to these environmental changes and also enhances its resilience to stress.

The role of bacteria as coral pathogens has dominated coral-microbiology research in recent years, due to the role of pathogenic bacteria in coral disease and bleaching, which has contributed to the global demise of coral reefs (Frias-Lopez et
al 2002). Currently, seven of thirteen diseases responsible for coral mortality have been linked to specific bacterial species, and include: aspergillosis, black band disease, white band disease type II, plague type II, white pox, yellow pox, and bleaching (Richardson 1998; Rosenberg et al. 2007).

The issue of how a microbial community shifts from beneficial bacteria to pathogenic bacteria and the mechanisms behind these shifts is now considered a key element in understanding the health of coral reefs (Bourne & Munn 2005). However, the ecological mechanism behind the establishment of the coral-bacteria association also remains unknown. Chemotaxis allows bacteria to improve their adaptation to the local environment by moving in response to chemical stimuli (Lux and Shi, 2004; Seymour et al. 2008), which may potentially play a role in coral-bacterial associations, e.g. through ‘settlement cues’ (Fig. ii) (Webster & Hill 2007).

Chemotaxis may also assist bacteria in finding specific coral niches, such as the CSM (Fig. i), and there is evidence that chemotaxis may be an important component in the pathogenicity of coral disease causing bacteria; as demonstrated by the infection of the coral *Oculina patagonica* by the bacterium *Vibrio shiloi* (Koren & Rosenberg 2006; Rosenberg et al 2007). Banin et al. (2001) suggests that zooxanthellae exudates contribute to the production of coral mucus, which *V. shiloi* is chemotactic to and only infects mucus containing zooxanthellae exudates. Neither the specific chemical attractants or chemotactic abilities of coral-associated bacteria have been examined, although there is evidence that marine bacteria can be highly chemotactic (Banin et al. 2001; Koren & Rosenberg 2006; Rosenberg et al 2007).

We propose that bacterial chemotaxis plays a key role in establishing the relationship between the coral and the bacterial consortia. To assess this theory, it is necessary to understand whether bacteria are attracted to coral products such as zooxanthellae extra-cellular secretions like amino acids and sugars, or a combination of both the host and the zooxanthellae’s chemical products. This study aims:
• To determine if the coral species *Pocillopora damicornis* has an elevated microbial community associated with it when compared to the surrounding seawater.

• To determine if bacteria are chemotactically attracted to coral and identify key chemoattractants.

• To assess the role of chemotaxis in structuring coral-bacteria associations under field conditions on Heron Island.

**Materials and Methods**

To assess 1) the spatial associations between coral-associated bacteria and their coral hosts and 2) the chemotactic behaviour of coral associated bacterial communities, both laboratory-based and field-based studies were conducted.

**1. Coral-Bacteria spatial associations**

*Aquaria Study:*

The scleractinian coral *Pocillopora damicornis* was chosen for laboratory studies, as the photo physiology is well characterised and it is sensitive to stress such as thermal bleaching and bacterial diseases (Baird et al. 2002; Hill et al. 2008). *Pocillopora damicornis* is a branching coral which occurs in small colonies throughout the tropics.

Colonies were collected during July 2009 from Heron Island lagoon, in the Capricorn Bunker Group on the Southern Great Barrier Reef, (152°06'E, 20°29'S) (Appendix, Fig. A3). The colonies were broken into nubbins in March 2010 following the methods of Davies (1984). Each individual nubbin was suspended in the aquarium with nylon fishing line attached to a rack. Nubbins were given six weeks recovery time after fracturing, before experiments began. Nubbins were maintained in a recirculating 5000 L tank with artificial UV sterilised seawater (carbonate 140 ppm and Aquasonic ‘Ocean Nature’ synthetic sea salt) at the University of Technology, Sydney. Salinity was maintained at 33 psu in reverse osmosis water and the temperature was maintained at 25 ± 0.5°C. Light was kept constant at 200 µmol
photons m$^{-2}$ s$^{-1}$ using 400 W metal halide lamps, and the corals experienced a 12:12 hours light–dark cycle. All nubbins were heterotrophically fed with *Artemia salina* twice weekly throughout the experiment.

Experimental treatments included three Coral Surface Microlayers (CSM) samples: healthy corals, diseased corals, light-excluded corals, and the control were surrounding tank water. For each treatment, four replicate nubbins were sampled five times, ($n = 80$). The healthy CSM corals had no visible sign of disease or bleaching, with tissue intact and polyps extended, as well as a healthy appearance in colour. The diseased CSM corals exhibited a migrating white-band, indicative of bacterial infection, which caused not only tissue degradation and sloughing, but also a bleached appearance. These disease characteristics are similar to the tissue necrosis associated with white syndrome or white disease (Ainsworth et al. 2007) as there was a clear lesion boundary and exposed skeleton (Appendix: Fig. A8). The light-excluded CSM corals were originally healthy corals, with no signs of disease. They were placed into a separate aerated tank, which was then maintained in complete darkness. The lack of light inhibited the zooxanthellae’s ability to photosynthesise and led to the expulsion of the zooxanthellae, causing a bleached appearance of the coral. However, these corals were sustained with *Artemia salina* as a heterotrophic food source.

Bacteria in the three CSM types and the surrounding tank water were each sampled using 1 mL syringes following the methodologies of Coles & Strathmann (1973); Ducklow & Mitchell (1979a); Coffoth (1990); Ritchie & Smith (1997); Kellogg (2004); Bourne & Munn (2005), where 500 µl sample was taken from immediately adjacent to the CSM. Samples were then fixed with glutaraldehyde (1% final concentration), before being frozen in liquid nitrogen.

*Heron Island Study:*

The experimental sites were located 80 km offshore at Heron Island (152°06'E, 20°29'S) in the Capricornia Bunker Group in the Southern Great Barrier Reef, Australia (Appendix: Fig. A3). Coral-associated water from several sites in Heron Island lagoon were described as tidal shallow water at a depth between 0.50 m – 2
m, with the presence of many coral colonies including the coral species, Pocillopora damicornis and Montipora digitata, which were sampled here. Syringe sampling was conducted adjacent to the coral CSM, as described previously.

For each of the two species, the CSM of three healthy coral colonies was sampled (500 µL) in triplicate with 1 ml syringes. For each of the two species, the CSM of three diseased colonies was also sampled in triplicate, these corals exhibited tissue necrosis as well as a bleached. A patch of open water, without coral was chosen to sample coral-free seawater nine times. Samples were fixed and frozen as described above.

Prior to analysis by flow cytometry, samples were quick-thawed and stained with SYBR Green I DNA stain [1:10,000] (Invitrogen Molecular Probes USA) and spiked with 1 µm fluorescent microspheres (Invitrogen Molecular Probes USA). Samples were analysed using a flow cytometer (BD LSR II USA) and bacterial populations were discriminated according to SYBR Green fluorescence and side-scatter (Marie et al. 1997). Flow cytometric data was analysed using Cell-Quest Pro (BD Biosciences). (see Appendix for details about the preparation of SYBR Green, fluorescent microspheres and FCM).

2. Chemotactic behaviour experiments

Laboratory Aquaria Study:

For analysis of chemotaxis of bacterial communities associated with corals maintained in aquaria at UTS, 1 L Schott bottles were used to collect water from the bulk water of the tank which contained corals, and was not collected adjacent to coral surfaces.

Heron Island Study:

Water was collected from immediately adjacent to Pocillopora damicornis colonies using 1 L Schott bottles at Heron Island Lagoon. To assess the chemotactic behaviour of bacterial communities, not associated with coral surfaces, two outer reef sites were selected to sample non-coral associated water for chemotaxis experiments only and were characterised by areas of deeper (10 m) open water,
and large patches of sandy substrate in deeper waters without any corals present in a radius of 10 m. The outer reef of One Tree Island (152°04'34"E, 23°28'62"S) south-east of Heron Island was also chosen to sample non-coral associated water for the zooxanthellae exudates and DMSP chemotaxis experiments. The Capricorn channel in between Heron Island and One Tree Island was also sampled using 1 L Schott bottles for more non-coral associated water for zooxanthellae and DMSP chemotaxis experiments only.

**Chemoattractants:**

A variety of chemoattractants were selected according to previous evidence of their existence in coral holobiont (Hellebust 1965; Von Holt & Von Holt 1968; Bell & Mitchell 1978; Meikle et al 1988; Johansen et al 2002; Raina et al 2009). The chemoattractants included amino acids (tryptophan, aspartic acid, casamino acid, lysine, valine, and threonine), sugars (arabinose, mannose, fucose and galactose), dimethyl sulphide (DMS) and dimethylsulphonopropionate (DMSP). All attractants were diluted in 0.2 µm filtered seawater (FSW) to a range of concentrations (1 µM - 10 mM). FSW was used as a control for each experiment.

We also examined the chemotactic response of bacteria towards the extracellular exudates from three clades of zooxanthellae. *Symbiodinium adriaticum* cultures CS-73, CS-156, and CS-159 were obtained from the CSIRO Micro algal Collection (CSIRO, Hobart, Australia). CS-73 is from an unknown clade and was isolated from a clam *Tridacna maxima* on Heron Island in 1978. CS-156 is from Clade C (Hill et al. 2009), and was isolated from the encrusting and massive coral *Montipora verrucosa* in Hawaii in 1983. CS-159 is also from an unknown clade and was isolated from a clam *Tridacna maxima* in Palau in 1983. Cultures were grown in 500 ml conical flasks containing 250 mL f/2 medium (Appendix) (Guillard & Ryther 1962; Guillard 1975) under 12:12 light:dark at 40 µmol photons m⁻² s⁻¹ at 25°C.

Every two days the photosynthetic parameter of effective quantum yield and PSII was determined for each zooxanthellae clade using Pulse Amplitude Modulation
Fluorometer with a Pocket PAM (Walz, Germany). Every four days 10 mL of cells were extracted using a serological pipette and filtered through a 0.2 µm milipore filter. The filtrate, containing the zooxanthellae extracellular products was then frozen prior to use as a chemoattractant. Filtrates from weeks 2 and 4 were used for chemotaxis experiments on Heron Island, and filtrates from all four weeks were used in laboratory aquaria. The response to f/2 growth medium was also measured as a control.

**Experimental Design:**
To examine the chemotactic responses of coral-associated and non-coral associated bacterial communities, a modified version of the capillary assay (Adler 1973) was employed, where 1 ml syringes were filled with 150 µl of each chemoattractant (Appendix Fig. A1). The syringes were then inserted into 100 ml vials filled with unfiltered seawater or tank water for 30 minutes. The diffusion of chemoattractants from syringes into the seawater suspension created a signal for chemotactic bacteria to respond to and migrate into the syringes. Experiments were conducted for a short time period (30 minutes) (see Appendix for time-series pilot study) to ensure that resultant bacterial concentrations in syringes were a product of chemotactic migration and not growth (also see Appendix for growth experiments confirming the lack of growth during this period). Following the 30 minute incubation period, samples were fixed with glutaraldehyde (1% final concentration) and frozen in liquid nitrogen prior to flow cytometric analysis, as described above.

**Data Analysis:**
Following cell enumeration analysis of flow cytometry data (Cell-quest Pro), bacterial concentrations were calculated (refer to Appendix) and the normality of data for both coral-bacteria spatial associations and chemotaxis experiments was tested using Levene’s Homogeneity of Variances. If these assumptions were not met log₁₀ transformations were performed. Following this, non-parametric tests (Kruskal-Wallis and Mann-Whitney U Tests) were used for post-hoc comparison for coral-bacteria spatial associations. ANCOVA (Analysis of covariance) was used for
chemotaxis experiments between water types (coral-associated water and non-coral-associated water). Our dependent variable was bacterial concentration, with independent quantitative and qualitative variables, such as chemoattractant, concentration and water type (Dyitham, 2003). The "covariate" in this study was concentration, and the inclusion of this accounts for some of the variability for which concentrations is responsible for within the dependent variable. The inclusion of a covariate can increase the statistical power, yet it can be limiting as it reduces the degrees of freedom. ANOVA was used for DMS, DMSP and zooxanthellae results within water types, as the ANCOVA indicated no differences between certain variables which could be ruled out in the ANOVA. All analyses were carried out using Minitab statistical software.
Results

1. Coral-Bacteria spatial associations

Laboratory Aquaria Study:

Mean bacterial concentrations in the Coral Surface Microlayer (CSM) of healthy corals were $6.0 \times 10^4 \text{ ml}^{-1} \pm 1.0 \times 10^4 \text{ SE}$ and were significantly ($P<0.05$) more abundant than the bacteria found in the surrounding water ($6.9 \times 10^3 \text{ ml}^{-1} \pm 2.0 \times 10^3 \text{ SE}$). The bacterial concentrations found in the healthy CSM were also significantly ($P<0.017$) greater than in diseased CSM’s, which had $6.0 \times 10^3 \text{ ml}^{-1} \pm 6.0 \times 10^2 \text{ SE}$ (Fig. 1). Concentrations were $3.7 \times 10^4 \text{ ml}^{-1} \pm 2.0 \times 10^3 \text{ SE}$ in the light-deprived CSM, which was significantly ($P<0.017$) greater than in the diseased CSM and surrounding seawater.

![Figure 1. Bacterial concentration per ml for healthy, diseased and light deprived coral surface microlayers of *Pocillopora damicornis* compared to bacterial concentrations in the surrounding tank water in the laboratory. *n* = 16. (Error bars are SE) *Significantly different to control, surrounding water*](image)
Heron Island Study:

In comparison to the laboratory studies, where aquarium water was used, concentrations of bacteria in the CSM retrieved from natural coral samples on Heron Island were higher, with an average of \(7.0 \times 10^5\) ml\(^{-1}\) ± 6.0 \(\times 10^5\) SE observed in healthy CSM’s of *Pocillopora damicornis*, and \(8.0 \times 10^5\) ml\(^{-1}\) ± 5.0 \(\times 10^4\) SE in *Montipora digitata*. However, no significant difference was observed in bacterial abundances between the healthy CSM and those of the surrounding sea-water for both *P. damicornis* and *M. digitata* (P=0.606 and P=1.01 respectively) (Fig. 2).

The Heron Island samples showed that there were significantly (P=0.006 and P=0.021) more bacteria in the CSM of diseased colonies of both *P. damicornis* and *M. digitata* when compared to the surrounding seawater (Fig. 2). The diseased CSM had an average of \(1.0 \times 10^6\) ml\(^{-1}\) ± 1.0 \(\times 10^5\) SE for *P. damicornis* and \(1.0 \times 10^6\) ml\(^{-1}\) ± 1.9 \(\times 10^5\) SE for *M. digitata* respectively, compared to the surrounding water which had a mean of \(8.0 \times 10^5\) ml\(^{-1}\) ± 6.6 \(\times 10^4\) SE. There were more bacteria associated with diseased CSM than there were of healthy CSM for both *P. damicornis* and *M. digitata* (P=0.002 and P=0.011 respectively) (Fig. 2).

![Figure 2. Bacterial concentrations per ml for healthy and diseased coral surface microlayers of *Pocillopora damicornis* and *Montipora digitata* compared to bacterial concentrations in the surrounding lagoon water at Heron Island. \(n=18\). (Error bars are SE) *Significantly different to control, surrounding water*](image_url)
2. Chemotactic behaviour experiments

*Sugars:* Bacteria associated with corals generally showed high levels of chemotaxis towards the sugars that were tested. The sugar which bacteria exhibited the highest levels of chemotaxis to from all water types (coral-associated water and non-coral-associated water) was arabinose ($P=0.0007$), although no significant difference between the tested concentrations of all attractants occurred ($P=0.296$) (Fig 3). In aquarium water, only galactose ($P=0.0188$) and arabinose ($P=0.005$) were significantly more attractive than the control (Fig. 3A). Furthermore, in aquaria samples, concentrations of bacteria attracted to sugars during the incubation period reached 6 times higher than background concentrations ($P=0.000$), with a maximum response to arabinose.

In all cases, the highest levels of chemotaxis towards sugars occurred in the coral-associated water from Heron Island ($P=0.000$). Bacterial concentrations from coral-associated water attracted to the sugars reached 50 to 176 times higher than background concentrations. Despite this, no significant difference was found between sugars and the control filtered seawater (FSW) ($P=0.726$) for coral-associated water.

In comparison, the chemotactic response of bacteria from non-coral associated water was significantly lower ($P=0.000$), with the maximum concentration of bacteria attracted to the sugars reaching only 5 times higher than what was found in background concentrations (Fig. 3c), with the response to arabinose the strongest overall ($P=0.000-0.001$). All concentrations of arabinose proved to be the only sugar to have a significant difference to the control in non-coral associated water ($P=0.003$) (Fig. 3c).
Figure 3. Ratio of bacterial chemotaxis to sugars of arabinose, mannose, fucose, galactose and the control filtered sea water to concentrations found in background at A) laboratory at UTS, B) coral-associated water from Heron Island lagoon, and C) non-coral associated water from Heron Island outer reef. n = 39. (Error bars are SE) *Significantly different (all concentrations) to control, FSW
**Amino Acids**: Bacterial chemotaxis towards amino acids showed a similar trend to that observed for sugars, with bacteria associated with corals from Heron Island showing significantly higher levels of chemotaxis than non-coral associated bacteria (P=0.000) (Fig. 4). All amino acids, especially threonine and lysine, invoked a significantly stronger chemotactic response than the FSW control (P=0.000 to 0.0272), except for tryptophan (P=0.2393), but there was no significant difference between the response to different concentrations (P=0.102).

There was a greater chemotactic response to amino acids by the aquarium bacteria than by the bacteria from non-coral associated water from Heron Island (P=0.000). Threonine was the strongest attractant, with bacterial concentrations reaching 29 times higher than background (Fig. 4a). All amino acids except for tryptophan (P=1.000), and casamino Acids (P=0.7718) were more attractive than the FSW control in the laboratory experiments, with threonine (P=0.000) and Aspartic Acid (P=0.0030) invoking the largest chemotactic response (Fig. 4a).

Chemotaxis towards amino acids was highest in the coral-associated water from Heron Island (P=0.000) (Fig. 4B). Here, bacterial concentrations responding to amino acids reached between 6 to 186 times higher than background, with a maximum significant response to tryptophan (Fig. 4b). Bacteria also showed significant chemotaxis responses to casamino acids, aspartic acid, and valine (P=0.000). There was no significant bacterial chemotaxis towards Lysine and Threonine (P=0.413 and P=0.070).

The bacterial response to amino acids from the non-coral associated water from Heron Island was significantly lower than the bacterial response from coral-associated water (P=0.000) (Fig. 4c). Only Lysine led to significant chemotaxis, with bacterial concentrations reaching 4.6 times higher than background (P=0.0019).
Figure 4. Ratio of bacterial chemotaxis to the amino acids of valine, lysine, threonine, aspartic acid, tryptophan, casamino acid and the control filtered sea water to concentrations found in background at A) laboratory at UTS, B) coral-associated water from Heron Island lagoon, and C) non-coral associated water from Heron Island outer reef. $n=57$. (Error Bars are SE) *Significantly different (all concentrations) to control, FSW
**DMSP and DMS:** There was a significant difference between water types \((P=0.000)\), where the highest levels of chemotaxis towards DMSP occurred in the coral-associated water from Heron Island \((P=0.000)\), and the lowest bacterial chemotaxis to DMSP was from non-coral associated water from Heron Island, as well as from aquaria (Fig. 5). Although there was no significant difference found between DMSP and FSW \((P=0.576)\), concentrations of bacteria in DMSP syringes reached 10 to 58 times higher than the background concentrations in coral-associated water on Heron Island (Fig. 5b).

The non-coral-associated water from Heron Island and laboratory aquaria were not significantly different from each other \((P=0.9980)\) (Fig. 5). There also proved to be no significant difference between DMSP and the control \((P=0.066)\), or between concentrations \((P=0.154)\). The bacterial response to DMSP from the non-coral associated water from Heron Island was significantly lower than the bacterial response from coral-associated water \((P=0.000)\) (Fig. 5c). However, there was no significant difference found between DMSP and FSW \((P=0.576)\).

DMS was significantly more attractive to bacteria in laboratory aquaria \((P=0.000)\); however, DMS was not tested on Heron Island. For aquarium bacteria, chemotaxis was the greatest towards the higher concentrations of DMS and DMSP (Fig. 6). DMS was a stronger attractant for bacteria \((p=0.000)\) than DMSP or FSW in aquaria at UTS (Fig. 6).
Figure 5. Ratio of bacterial chemotaxis to DMSP and the control filtered sea water to concentrations found in background seawater at A) coral-associated water from Heron Island lagoon, B) non-coral associated water from Heron Island outer reef (there is data in the graph, but the y scale is too large to see this) C) non-coral-associated water from One Tree Island and D) Non-coral-associated water from Capricorn Channel. n=16. (Error bars are SE)

Figure 6. Ratio of bacterial chemotaxis to DMS, DMSP and the control filtered sea water to concentrations found in background in aquaria at UTS. n=7. (Error bars are SE) *Significantly different (all concentrations) to control, FSW
*Extracellular products from cultured zooxanthellae:* Bacteria associated with corals from Heron Island exhibited the highest levels of chemotaxis towards zooxanthellae extracellular products than bacteria from non-coral associated water or from aquaria (Fig. 7b). A significant difference was found between clades and water type (P=0.000), but not for week (P=0.535) for all water types. Bacteria from all water types exhibited chemotaxis towards all clades of zooxanthellae exudates (P=0.0002 to 0.0494) over the control, filtered seawater. Bacteria from aquaria and all non-coral water except from the Capricorn channel exhibited the least amount of chemotaxis to the exudates (P=0.000).

Highest levels of chemotaxis towards zooxanthellae exudates occurred amongst coral-associated bacteria from Heron Island (P=0.000) (Fig. 7b). Concentrations of bacteria responding to strain CS-156 reached 51 times higher than background (Fig. 7b). CS-73 was the most attractive exudate to bacteria from non-coral water, as it had a concentration of 1.3 times higher than background concentrations with a maximum mean bacterial count at $3.9 \times 10^5 \text{ ml}^{-1} \pm 8.9 \times 10^4 \text{ SE}$. (Fig. 6b).

Significant chemotaxis was found for all zooxanthellae clades in aquaria at UTS (P=0.000) (Fig. 8), and there was a difference between weeks (P=0.030). Bacteria from aquaria exhibited chemotaxis towards weeks 3 and 4 in preference over f/2 media and FSW.

Throughout the study all three clades of zooxanthellae had healthy Effective Quantum Yields ($\Phi_{PS II}$) between 0.53 and 0.78 (Fig. 9).
Figure 7. Ratio of bacterial chemotaxis to exudates from three zooxanthellae cultures, CS-73, CS-156, and CS-159. With f/2 media and filtered sea water as the control to concentrations found in background at A) coral-associated water from Heron Island lagoon, B) non-coral associated water from Heron Island outer reef (there is data in the graph, but the y scale is too large to see this) C). non-coral-associated water from One Tree Island and D) Non-coral-associated water from Capricorn Channel. n = 32. (Error bars are SE)

Figure 8. Ratio of bacterial chemotaxis to exudates from three zooxanthellae cultures, CS-73, CS-156, and CS-159. With f/2 media and filtered sea water as the control to background concentrations in the laboratory at UTS. n = 14. (Error bars are SE)
Figure 9. Effective quantum yield of PS II of three zooxanthellae cultures throughout the study, weeks 1-4 are when exudates were sampled. \( n=3 \)

Discussion

1. Coral-bacteria spatial associations:

We observed strong coral-bacteria spatial associations in laboratory samples from aquaria, which are consistent with previous research (Ducklow & Mitchell 1979; Paul et al. 1986; Ritchie & Smith 1997; Koren & Rosenberg 2006), where elevated bacterial abundance was associated with the coral surface microlayer (CSM) when compared to the surrounding water. Ritchie & Smith (1997) suggested these results may be a sign of distinct microbial communities being associated with specific coral species (Ritchie & Smith 1997). However, in the field on Heron Island, although bacterial abundance found in healthy CSM’s were equal to the surrounding water for both *Pocillopora damicornis* and *Montipora digitata*, the counts were more consistent with literature at \(10^5\text{-}10^6\text{ ml}^{-1}\), and thus higher than what was found to be associated with corals kept in aquaria. The similarity of bacterial concentration between the surrounding seawater and the healthy CSM in the field may be a result
of low mucus production and low stress, supporting only a small population of bacteria in the healthy CSM (Vacelet & Thomassin 1991).

The difference in bacterial concentrations between the laboratory aquaria and Heron Island is perhaps evidence of the selection of coral-microbial association by environmental conditions which the coral is exposed to. Earlier research confirms our results that coral mucus in the field has a higher abundance of microorganisms when compared to mucus from coral kept in a laboratory aquaria (Kooperman et al 2007). Molecular studies have also found little similarity between reef water and coral-associated bacterial species and the interaction between these two communities remains unknown (Fias-Lopez et al. 2002; Rowher et al. 2001; Klaus et al. 2005; Lampert et al 2008).

High bacterial abundances associated with diseased corals from Heron Island are consistent with previous research, which suggests that bacteria in coral mucus respond to the host’s stress by increasing in abundance (Ducklow & Mitchell 1979). In our study, bacterial abundance in a healthy coral was almost half that observed in a diseased CSM on Heron Island. Symptoms of this disease appear to be similar to the tissue necrosis of white syndrome or white disease (Ainsworth et al. 2007) as there was a clear lesion boundary and exposed skeleton. However, for corals kept in laboratory aquaria, results imply that more bacteria are associated with disease-free CSM’s. The low bacterial abundance found associated with diseased corals in aquaria may be due to differences in the bacterial communities (Kooperman et al. 2007), different stages of the disease cycle in aquaria compared to that on Heron Island, or different diseases, as we could only speculate on diseases, which were sampled on Heron Island and in laboratory aquaria. The nubbins in laboratory aquaria were rather small (approximately 3 cm x 3 cm) when compared to a whole colony in the lagoon (approximately 20 cm x 20 cm), perhaps providing a larger surface area for microbial settlement, infection and migration.

Our findings in the field on Heron Island also indicate that bacterial abundances are comparable across the two species tested, *P. damicornis* and *M. digitata*. These results contradict previous research, which suggest that different species of coral
maintain varying populations of bacteria in their CSM’s, where some species have high bacterial populations, and others have less (Ducklow & Mitchell 1979). Our results demonstrate there is a clear dynamic mechanism behind coral-bacterial spatial associations, which could be driven by the ecology and the behaviours of the bacterial consortia associated with the coral. We investigated the potential role of chemotaxis in establishing this association between corals and bacteria in both experimental aquaria, in the field on Heron Island, One Tree Island and Capricorn channel.

2. Chemotaxis behaviour experiments:
Our results clearly suggest that chemotaxis is strongest amongst bacteria from coral-associated water from Heron Island, in comparison to bacteria from non-coral-associated water, both in the field and laboratory aquaria. Our results from aquaria experiments emphasise this finding, as water was collected from the bulk tank water and not next to coral surfaces. This indicates that chemotaxis is more important to bacterial communities associated with coral surfaces. This pattern occurred for all chemoattractants tested, and confirms that these compounds are likely to be present and ecologically important within the coral holobiont. These findings also confirm previous research that chemotaxis is an important behavioural mechanism for bacteria (Lux and Shi, 2004; Seymour et al 2008). More importantly however, our results suggest that coral-associated bacteria may use chemotaxis to establish a relationship with corals through the sensing of coral-associated chemicals. Ritchie et al. (1996) implies the establishment of ‘normal’ microbiota in the CSM is through the nutrition and consequently the metabolic by-products of the coral and zooxanthellae. But first the microbial consortia must establish an association with the host and zooxanthellae, and through these chemotaxis experiments our results shed new light on what causes this establishment. Furthermore, previous research has indicated this establishment of microbiota varies from species to species of coral, which leads to the development of a microbial community specifically characteristic of that species (Ritchie et al. 1996a), and chemotaxis could indeed be an important mechanism behind this partitioning of microbial communities to different corals or niches within a coral.
The chemotactic response to several sugars was studied in our experiments, with sugars selected in accordance with the results of Meikle et al (1988), where it was shown that mucus samples contained high levels of fucose, arabinose, mannose and galactose. Sugars are an important energy source for bacteria, as they are important for carbohydrate metabolism (Kline et al. 2006) and our results suggest chemotaxis towards sugars may be important in forming coral-bacteria associations in coral reefs.

In our experiments, arabinose proved to be the most attractive sugar amongst all water types (coral-associated and non-coral-associated water). Arabinose originates from zooxanthellae, and previous studies have shown it is a major constituent of coral mucus composition (Ducklow & Mitchell 1979; Meikle et al. 1988; Banin et al. 2001; Wild et al. 2004). Our findings show that bacteria from non-coral associated water were chemotactically attracted to arabinose, which is released by zooxanthellae, and it could be argued that it is through the zooxanthellae’s excretion of arabinose that bacteria possibly establish an initial association with coral through chemotaxis. From previous studies (Kline et al. 2006) and from our results, it could be argued that the zooxanthellae can regulate the bacterial consortia as it produces sugars, in turn controlling Dissolved Organic Carbon (DOC) levels in the CSM. Kline et al (2006) explained that the addition of organic sugars increased DOC levels in the CSM, and it is possible that this increase in DOC could attract bacteria, causing chemotaxis, as shown in our results. It has been suggested that the bacterial consortia inhabiting the CSM are carbon limited, and this could be the case for coral-associated bacteria, as it has been shown the addition of sugars assists microbial degradation of previously unavailable carbon sources (Kline et al. 2006). Interestingly, it has been demonstrated that some coral mortality is due to the elevated DOC levels promoting a population explosion in the bacterial consortia in the CSM (Kline et al. 2006). Possible causes of death are from the increased oxygen uptake by more bacteria, predation by these bacteria, as well as poisoning by their metabolites (Kline et al. 2006).
We also found clear chemotactic responses to several amino acids that are expected to occur in the holobiont. In a comprehensive study of the secretion of organic compounds by zooxanthellae, Von Holt and Von Holt (1968) found that all zooxanthellae excrete either glutamic or aspartic acid. Aspartic acid was chosen in our study as it is the predecessor to essential amino acids including methionine, threonine (Appendix) and lysine in bacterial metabolism (Lehninger et al. 2000), which were also included. Meikle et al (1988) examined the structure of mucus from six species of coral and also found aspartic acid, threonine, valine, and lysine in the mucus contents, as well as tryptophan, which was thus also selected for this study, along with casamino acids, which is an equimolar mixture of all the essential amino acids.

Like sugars, our results show strong bacterial chemotaxis towards amino acids from coral-associated water, and as amino acids are important growth substrates for bacteria, this could be the driving mechanism behind chemotaxis towards amino acids. Our results relate to Adler’s (1973) capillary assays, which identified amino acids as bacterial attractants (Englert et al. 2009). Mesibov and Adler (1972) demonstrated that the enteric bacterium *E. coli* exhibited chemotaxis towards amino acids such as aspartate, methionine, serine, and threonine. However, *E. coli* was not attracted to histidine, lysine, tryptophan or valine (Mesibov & Adler 1972).

The examinations of the specific chemical attractants and chemotactic abilities carried out by Mesibov and Adler (1973) have not yet been conducted for coral-associated bacteria, yet, our results shed new light and show that coral-associated bacteria were highly chemotactic to chemicals, which *E. coli* found to be a repellent. These included valine and lysine, which indicate that coral-associated bacteria are different to *E. coli*, and possibly have different metabolic needs.

Results show strong bacterial chemotaxis towards sugars, and the driving force behind this may be for the synthesis of amino acids, where previous research (Fitzgerald & Szmant 1997) has demonstrated that bacteria convert sugars from the zooxanthellae into amino acids. In turn the coral holobiont utilises these amino acids. Our findings show that bacteria from coral-associated water exhibited the greatest chemotaxis towards amino acids. These bacteria showed significant
chemotactic response towards essential (valine, casmino acids) and non essential amino acids (aspartic acid, casamino acids) (Lehninger et al. 2008), which implies that these bacteria are attracted to a combination of amino acids synthesised by the zooxanthellae and coral, and perhaps to chemicals synthesised by other bacteria in the holobiont. Aspartic acid is non-essential, so it is synthesised from the corals metabolic pathways. Valine is an essential amino acid synthesised in plants, so is likely to be of zooxanthellae origin in coral-associated water. Our results show that coral-associated bacteria are chemotactic to aspartic acids indicating this amino acid could be essential to bacteria in the CSM, as they could use it to synthesise essential amino acids for their metabolic needs.

As well as there being a difference in the types of amino acids bacteria responded to, there was a difference in the strength of the response. Our study shows that threonine and aspartic acid caused the largest chemotactic responses in laboratory aquaria, where as bacteria from non-coral-associated water from Heron Island were only chemotactic towards lysine and tryptophan and reached a maximum concentration of 4.6 times higher than background. Aspartic acid and valine were the most attractive amino acids in coral-associated water from Heron Island, which reached bacterial concentrations between 6 to 186 times higher in comparison to background water. These findings imply that bacteria are chemotactic towards different amino acids in different environments and that bacteria associated with corals are more chemotactic towards amino acids than non-coral-associated bacteria, where perhaps bacteria associated with corals rely more upon amino acids for nutrition. It could be argued that bacteria from different environments have different metabolic needs, or that perhaps it is the environment, which dictates what chemicals are available, and hence are attractive to bacteria.

Environmental conditions may possibly dictate and regulate the bacterial consortia associated with coral as demonstrated by Klaus et al (2005), where it was noted that different reef conditions impacted differently upon the microbial population structure of coral. It was also suggested that the bacterial consortia associated with coral can be controlled directly through the environment, or indirectly through the same environmental conditions affecting the coral and zooxanthellae physiological
condition (Klaus et al. 2005). From our study it was shown that amino acids were more attractive to bacteria from coral associated water. It has been established that during times of stress, coral increase mucus production in the CSM (Segal & Ducklow 1982; Ritchie et al 1996a), and this could also apply to different environmental conditions which coral is exposed to. Coral thrive under narrow optimal environmental conditions, where changes in these conditions can affect temperature, pH, salinity, increased sediment loading, and elevated nutrients, and in turn cause stress to the coral (Frias-Lopez et al. 2002; Klaus et al. 2005). Under different environmental conditions and at times of stress, it is possible that coral could secrete different chemoattractants to attract specific bacteria to help maintain homeostasis, or conversely, bacteria could be attracted to certain chemoattractants which signal that coral is vulnerable and ideal for pathogenic infection.

Dimethylsulfiniopropionate (DMSP) was also highly attractive to coral-associated bacteria. DMSP is an important source of carbon and reduced sulphur for marine bacteria (Howard et al. 2006), is found in high quantities in coral reefs, and our results show that coral-associated bacteria are chemotactic towards DMSP. There are two pathways for bacterial degradation of DMSP, one where the organic sulphur is assimilated into proteins (Raina et al. 2009), and secondly where DMSP is converted to dimethyl sulfide (DMS), which is involved in the process of cloud formation (Raina et al 2009). The majority of DMSP on coral reefs is produced by zooxanthellae and is excreted into the surrounding CSM, where it is degraded by marine bacteria transforming a portion into DMS (Raina et al. 2009). Hence, zooxanthellae are responsible for bacterial chemotaxis towards DMSP and not the coral. Yet, without the coral providing somewhere for the zooxanthellae to reside, there would be no production of DMSP by the zooxanthellae into the CSM, and bacterial chemotaxis would not occur. Although DMSP is of zooxanthellae origin, the symbiotic relationship between the coral and zooxanthellae is a significant mechanism behind chemotaxis as it enables bacteria to establish an association with the holobiont.
DMSP is an important nutrient for coral-associated bacteria, hence causing chemotaxis, and in turn influences the structure of the bacterial communities, affecting the health of corals and the reef as an ecosystem (Raina et al. 2009). Our results show that DMSP is also an important behavioural cue for coral-associated bacteria and may act as a significant driver of coral-bacteria associations. Our additional observation of strong chemoattraction towards DMS in our laboratory aquaria study adds further support to the hypothesis that bacteria play an important role in sulphur cycle with the coral holobiont and across coral reef ecosystems (Raina et al 2009).

After observing chemotaxis to a suite of important organic substrates that occur in the coral holobiont, we examined the chemotactic responses to zooxanthellae extracellular products, which represent a more complex, and potentially more holistic, chemotactic signal (Hellebust 1965; Bell & Mitchell 1972; Johansen et al 2002). Exudates from all three clades of zooxanthellae were more attractive to bacteria than the control filtered seawater from all water types, where the highest levels of chemotaxis towards zooxanthellae exudates occurred amongst coral-associated bacteria from Heron Island. Our results show that bacteria associated with corals are highly chemotactic to zooxanthellae exudates, which suggests that zooxanthellae exudates are an important chemical cue for marine bacteria to establish an association with coral.

Similarly bacterial communities from the laboratory aquaria exhibited significant chemotaxis towards all three clades of zooxanthellae exudates in preference to the control. These findings suggest that the bacteria in the aquarium are highly attracted to zooxanthellae exudates. Also, bacteria were more attracted to exudates from weeks 3 and 4 in preference to the controls, and this demonstrates that it is possible that the zooxanthellae excretions became more attractive to bacteria once growth ceased, and decomposition began as suggested by Bell and Mitchell (1972). Recent research using marine bacteria has shown that they exhibit a chemotactic response to the nutrient patches caused by cell lysis of algae (Bell & Mitchell 1972; Johansen et al. 2002). This nutrient patch only lasts for a short period of time where marine bacteria’s motility and swimming speed enhances its
ability to exploit such patches, in turn leading to high bacterial abundances, which are independent of the surrounding concentrations (Johansen et al. 2002). However, Effective Quantum Yield of $\Phi_{\text{PS II}}$ obtained from PAM fluorometry for all three clades shows throughout the study, the zooxanthellae cultures were all healthy with Effective Quantum Yield of $\Phi_{\text{PS II}}$ between 0.55 and 0.88. This contradicts work of (Bell & Mitchell 1972) as cell lyses was not occurring at time of sampling the exudates. For the most attractive weeks, 3 and 4, the Effective Quantum Yield of $\Phi_{\text{PS II}}$ had a minimal range between 0.64 and 0.65, suggesting the bacteria are highly attracted to exudates from zooxanthellae which are healthy in a narrow range.

It must also be considered that only a small number of chemoattractants expected to be associated with coral mucus were tested in this study, which leaves opportunity for future research to determine what compounds from coral mucus are positive chemoattractants for coral-bacteria associations.

Results from this study indicate that bacterial behaviour, like chemotaxis plays an important role in contributing to the highly structured nature of microbial consortia associated with corals. The motivating mechanism behind bacterial association with coral may differ for beneficial and pathogenic bacteria, where motility and chemotaxis are very expensive metabolic processes. Despite this, bacteria benefit from this association with coral, whereby they improve their access to elevated nutrient patches for growth, which could be derived from the holobiont, or from organisms floating past in the water column that become trapped in the CSM in otherwise oligotrophic waters. Bacteria may also colonise the CSM, leading to the creation of a biofilm attracting other bacteria (Witzany 2010), or migrate to other coral niches such as the gastrodermal cavity or skeletal layer (Rosenberg et al. 2007). Pathogenic bacteria may search for coral that are stressed to colonise, infect and cause disease, or lay dormant until environmental conditions are favourable for infection, which has already been demonstrated for the bacterial infection of _Oculina patagonica_ by _Vibrio shiloi_ (Banin et al. 2001; Koren & Rosenberg 2006;
Rosenberg et al. 2007). Future research will undoubtedly identify the key mechanisms driving the uncharacterised pathogenic infection of corals, and we expect chemotaxis will play an important role.

As coral-bacteria associations involve the holobiont as well, the coral and/or zooxanthellae may be able to regulate the population and abundance of the microbial consortia through the chemicals they release. This behaviour means that coral’s microbial biota may be enhanced and altered according to what chemicals it releases, so potentially chemotaxis could play an important role in the probiotic hypothesis, because corals may alter the chemicals they release to actively attract bacteria with specific chemotactic abilities. Our study shows that many of the chemoattractants bacteria responded to were of zooxanthellae origin; however, it must be kept in mind that without the coral providing somewhere for the zooxanthellae to reside there would be no production of these chemoattractants into the CSM, and bacterial chemotaxis would not occur. Hence, the symbiotic relationship between the coral and zooxanthellae is a significant mechanism behind chemotaxis as it enables bacteria to establish an association with the holobiont.

We have demonstrated the relationship between coral, bacteria and zooxanthellae is not closed to the environment and, we suggest the ability of the coral and zooxanthellae to control the microbial consortia may only be relevant during times where environmental conditions are favourable for the coral. Environmental conditions can have a direct or indirect impact on the bacterial consortia associated with corals (Klaus et al. 2005). If the holobiont’s physiological condition is compromised by changes in environmental conditions, such as increased temperature or pH, the holobiont relationship might swing in favour to pathogenic bacteria (Klaus et al. 2005). This may result in a change, reduction or complete loss of chemicals produced, which would ultimately affect not only the resident bacterial consortia, but also bacteria attracted to the coral.
Conclusions

Our results demonstrate there are high bacterial abundances associated with the CSM of P. damicornis, and this can be influenced by the health of the holobiont, as well as the environment in which it is exposed to. High bacterial abundances associated with diseased corals suggest that bacteria in coral mucus respond to the host’s stress by increasing in abundance. There is a dynamic mechanism behind these coral-bacteria spatial associations, which could be driven by the ecology and the behaviour of the bacterial consortia associated with the coral, or by the symbiosis of the coral and zooxanthellae.

Our research demonstrates that coral-associated bacteria may use chemotaxis to establish a relationship with corals through the sensing of coral-associated chemoattractants, as our results show that chemotaxis was strongest amongst bacteria from coral-associated water from Heron Island. This indicates that chemotaxis is more important to bacterial communities associated with specific coral habitats than in open water or in aquaria. This pattern occurred across all chemoattractants tested, and suggests that these compounds are likely to be important to the holobiont, as well as the driving mechanism behind chemotaxis. Our research indicates that coral mucus, where the composition is contributed by both the coral and zooxanthellae is attractive to bacteria.

Ongoing research in coral microbiology will undoubtedly reveal important insights into the relationship between corals and bacteria, and the mechanisms behind the establishment of this relationship. Of particular interest and concern will be the characterisation of chemoattractants used by pathogenic bacteria to locate, inhabit and infect corals, causing disease. Just as relevant is the continuing research into what constitutes as ‘normal’ bacterial consortia associated with healthy corals and how they differ to pathogenic bacteria. Whether or not particular chemoattractants originate from the host coral or from zooxanthellae, and how the environment influences the interactions within the holobiont will prove to be exciting future research. A better knowledge of these processes, which directly influence both the ecology of coral associated bacteria and the way they interact with their coral host,
will help us to improve our understanding of the underlying mechanisms structuring the composition and function of bacteria within coral reefs.
References


BD LSR II Users Guide (2007) BD Biosciences, San Jose, CA, USA


Davies, P. S. (1984). The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs. 2: 181-186


Lilley, R., M., Ralph, P., J., Larkum, A., W., D. (2010). The determination of activity of the enzyme Rubisco in cell extracts of the dinoflagellate alge Symbiodinium sp. by


